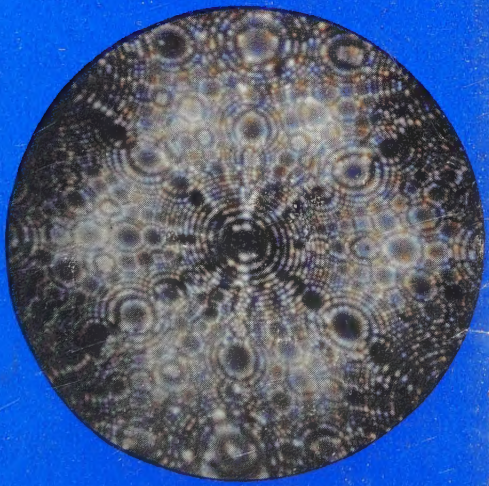
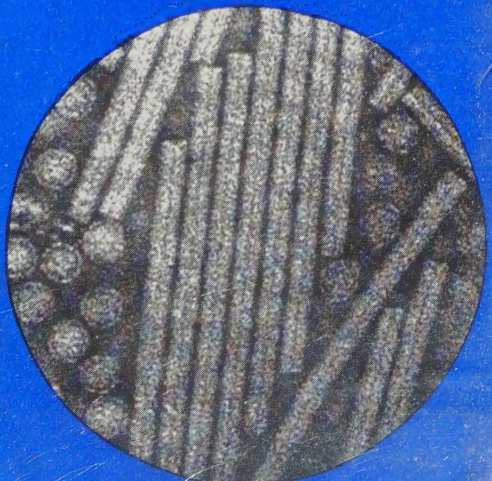
V.E.Cosslett
Reader in Electron Physics
University of Cambridge

Modern Microscopy





Exploration of the microstructure of Nature has made great strides in the last twenty years or so. The light microscope, developed over the previous three hundred years, shows us a great deal of the detailed architecture of plants and animals. The electron microscope, which only became a practical instrument for research in the 1940's, has extended our limits of vision by as much again in one-tenth the time. A whole new microscopical world has been opened up, revealing an undreamt-of ultra-fine structure in cells and viruses, and even within metals and minerals. At the same time, the scope of the light microscope has been greatly widened by the invention of the phase-contrast and interference techniques, which make it possible for the first time to look at living material in the course of growth and development.

It is the aim of this up-to-date book by a great authority to show something of what the new microscopes have added to our understanding of the world around us and also to make clear the limitations of the older methods of microscopy and the principles of the newer methods.

The many plates give some idea of the marvellous wealth of structural detail lying beneath the surface of the everyday world. They have been drawn from a wide variety of sources, in France, Germany, Japan, the U.S.A., and the Cavendish and other laboratories in Cambridge.

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V. E. COSSLETT, Sc.D

Reader in Electron Physics
University of Cambridge

Based on the 131st course of
Royal Institution
Christmas Lectures

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TO

Sir Lawrence Bragg, F.R.S.,
Director of the Royal Institution,
1953–1966, as some acknowledgement of
his encouragement and practical support
in my own adventures into microscopy.

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PREFACE

'Big fleas have little fleas upon their backs to bite 'em,
And little fleas have lesser fleas . . . and so ad infinitum.'

Exploration of the microstructure of Nature has made great strides in the past twenty years or so. The light microscope, developed over the previous 300 years, shows us a great deal of the detailed architecture of plants and animals. The electron microscope, which only became a practical instrument for research in the 1940's, has extended our limits of vision by as much again in one-tenth the time. A whole new microscopical world has been opened up, revealing an undreamt of ultra-fine structure in cells and viruses, and even within metals and minerals. At the same time the scope of the light microscope has been greatly widened by the invention of the phase-contrast and interference techniques, which make it possible for the first time to look at living material in the course of growth and development.

It was the aim of these Christmas Lectures, the 131st course given at the Royal Institution, to show something of what the new microscopes have added to our understanding of the world around us. Not simply as another display of the 'Wonders of Nature', but attempting also to make clear the limitations of the older methods of microscopy, and the principles of the newer methods. A certain amount of mathematics was consequently unavoidable, because this, like any other branch of science, involves quantitative relationships. Nothing more than elementary algebra and geometry was included, however, most of it being clarified by diagrams and demonstrations. Although the reader of this written version of the lecture has to forego the demonstrations, he has the possibility of going over the diagrams and arguments at his leisure. And if he feels that mathematics is not for him, he can take the results for granted and turn to the record of the new knowledge set out in the micrographs. Many more than are reproduced here were shown during the six lectures; the publishers have been very generous in allowing me to include these thirty-two

plates. Those who may like to know more about the implications of the new microscopy for biology will find an outline in *The Microstructure of Cells: an Introduction for Sixth Forms*, by S. W. Hurry (London, John Murray).

The pictures shown here should convey some idea of the marvellous wealth of structural detail which lies beneath the surface of the everyday world. But it is only possible to appreciate their smallness of scale by looking through a microscope oneself. Very few will have access to an electron microscope, but anyone can buy a magnifying glass and many should be able to lay hands on a microscope. The aims of the lectures and of this book will have been achieved to the extent that listeners and readers are moved to see things for themselves. A light microscope is not expensive, compared with a good watch or bicycle. But to get the most out of it, some care and understanding is needed; a good guide to consult is *The Use of the Microscope*, by R. Barer (Oxford, Blackwell).

V. E. C.

Postscript: Various pressures, mostly connected with microscopy, having delayed the preparation of this book, the opportunity has been taken to bring it up-to-date, particularly in respect of the rapid progress in electron microscopy and field-ion microscopy between 1960 and 1965.

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CHAPTER

1

THE MICROSCOPICAL WORLD

The Scope of the Microscope

BENEATH THE EVERYDAY appearance of ordinary things there lies a complicated and fascinating web of microscopical structure. That plants and animals have a detailed architecture is well known and the study of it belongs to biology. That rocks, stones, even metals, similarly contain fine structure is less generally appreciated. It is the task of microscopy to search out and lay bare this inner complexity, and if possible to relate it to the functions of the organism or the mineral. In biological studies, the anatomist and the physiologist rely on the microscope in one or other of its forms to aid them in investigating the way in which animals are made, live, develop and function. In mineralogy and metallurgy the work of recent years, especially with the electron microscope, has begun to show that inorganic matter also has a 'life' of its own. Metals and rocks are formed in conditions that can be specified, and may subsequently be changed by variation of temperature, pressure or other stress. Under the microscope we find corresponding changes in microstructure, which can be used to deduce, for instance, the probable lifetime of a turbine-blade or the state of the Earth's crust in which certain rocks were formed.

More fundamentally, the increasing power of modern microscopes is reaching down through ever finer levels of detail to the final laying bare of atomic structure. We all know, in abstract terms, that matter is built up from individual atoms of a limited number of elements. The light microscope, however, stops short by a very wide margin of revealing the size and arrangement of atoms—essentially because the wavelength of light is many thousand times bigger than an atom. The

electron microscope can in principle show detail down to the size of the electron itself, which is much smaller than the atom. Very recent developments give us the possibility of displaying atomic networks and, in a few special metals, even the atoms themselves. Organic substances are more difficult to investigate, the atoms being arranged with less regularity, but even here the electron microscope has been able to show the size and shape of many of the macro-molecules, composed of many thousands of atoms, and how they are twisted together to form chromosomes, muscle fibres and viruses.

In short, the new microscopy has made possible the systematic investigation of the structure of matter in the size range which runs from the atomic-molecular level up to the working limit of the light microscope. At the same time, new ideas and new technical developments have greatly enlarged the versatility of the light microscope itself. In particular, the phase-contrast microscope and the interference microscope now allow us to observe living cells, whereas in the ordinary microscope their internal detail is hardly visible owing to lack of contrast. The application of ciné techniques came along just at the right time, so that many of the processes of growth and division in cells have now been recorded in visible light. The still newer flying-spot method is further extending the range of optical microscopy, even to the automatic counting and size-grading of particles such as blood-cells.

In subsequent chapters the different methods of microscopy will be explained in detail, from the simple lens and the eye itself up to the electron and X-ray microscopes. Certain physical principles determine both the possibilities and the limitations of each type of microscope. Each has a definite place in the battery of research tools which the scientist uses in his exploration of the secrets of Nature or the technologist in his efforts to exploit them for the benefit of mankind. Examples will show the range of usefulness of the different methods of microscopy. But here, as in every branch of science, we find at once that we need scales of measurement for giving quantitative expression to our findings, we must have microscopic units for specifying the minute dimensions in which we have to move.

The Great Range of Microscopical Detail

To start with, we have to realise the great range of the microscopical world. It extends downwards in size, from human dimensions, by almost as many steps as in astronomy we go upwards to the scale of the solar system. The ratio of the height of a man to the radius of an atom is about 20 thousand million (or 20×10^9), whereas the ratio of the Sun's distance to the height of a man is about 70×10^9. It is almost impossible to picture such a huge distance as that of the Sun from the Earth, so let us confine our comparison to the Earth itself and take a golf ball as our unit. The golf ball is as much larger than the atom as the Earth is larger than the golf ball. Even the size of the Earth is difficult to comprehend; a useful exercise is to think of the longest journey one has ever made and then try to estimate what fraction of the Earth's surface this covered. In still more comprehensible terms, everyone will have a good idea of how much three miles is in distance and of the size of a flea (about 1 mm.). A flea is bigger than an atom in the same degree as three miles is bigger than the flea. Otherwise put: if everything were to be enlarged five million times, an atom would be as big as a flea is now, but the flea would be three miles across—and could easily jump the Channel.

Another way of getting an impression of the vast range of microscopical detail is to consider how many steps of subdivision are needed to reduce a common object to atomic size. Suppose that we start with a piece of wire just 1 inch long and cut it in half, in half again and so on. It would take 26 successive 'halvings' (supposing we could go on making finer and finer cuts) until the last cut gave us two atoms. In science it is usual, of course, to use the decimal system and work in powers of ten. So let us now suppose that we cut off one-tenth of our one inch wire, then one-tenth of this piece, and so on. It would still take 8 steps before the last cut of one-tenth proved to be an atom. In other words, there are about 100,000,000 atoms (or 10^8) to the linear inch.

In area and volume the relations are greater by the square

and cube of these numbers respectively. If we start with a cinema screen 20 feet square, for instance, and reduce it in the scale of ten, in 4 steps we reach a spot about $\frac{1}{40}$ inch square, roughly the size of a full stop on this page. It would still need between 6 and 7 more steps to reach the cross section of an atom; 6 steps gives an area in which ten atoms would fit. Or in terms of volume: if 10^8 atoms go to the linear inch, then a 1 inch cube will contain 10^{24} atoms, i.e. one million million million million.

The point of these examples is not to dazzle you with noughts but to convey some impression of the extraordinary smallness of atoms and molecules (which are groups of atoms more or less firmly bound together), and therefore of the great range and variety of patterns and structures which can exist between the atom and the dimensions of everyday objects. To say that an atom is one-million-million-million-millionth of a cubic inch does not by itself convey a very clear picture of how small it is. It becomes more comprehensible when we see that the Earth is made up of as many cubic inches as there are atoms in a single cubic inch. The variety of things on the surface of the Earth is enormous and their size range equally so, from a blade of grass to a continent. We may be sure that there is a similar multiplicity of structural detail within the Earth itself. As great a range as this, but now in descending order of size, exists within our cubic inch of matter before we come down to its component atoms. The microscopical world has an almost infinite richness of detail. With the new methods of microscopy we now begin to understand how it is built up in structures of increasing complexity, stage by stage from the molecule to the everyday object. Examples of the different sorts of architectural detail found in the successive size ranges are given later in this Chapter, taken from both biological and metallurgical specimens.

Microscopical Units of Measurement

In the metric system the standard of length is the metre and the ordinary unit of measurement is the centimetre. For defining a scale for microscopy we start with the millimetre. One *micron* is one-thousandth of a millimetre. It is denoted by the

Greek letter μ, pronounced 'mu', or sometimes by μm. The plural should be 'micra' but English usage is almost always 'microns'. A thickish hair is about 100 μ thick (0·1mm.), a sheet of typing paper about half as thick. A large blood cell is rather less than 10 μ across, and is easily seen with the light microscope. Bacteria are about 1 μ across, and need quite a good microscope even when they are stained red or blue to make them visible. The wavelength of yellow light is about 0·5 μ.

For measurements of everyday objects we normally use a ruler. In microscopy we prefer to use a 'graticule', or two-dimensional ruler, made by ruling very fine parallel lines in two directions at right angles on a glass plate so as to form a square grid pattern. This is clearly much more practical than trying to use a linear micro-ruler, and enables us to find the size of objects in any part of the field of view of the microscope. It is difficult, though not entirely impracticable, to rule lines 1 μ apart so as to give a graticule of 1,000 lines per mm. or 25,400 lines per inch (1 in. = 25·4 mm.). Graticules of 10 to 100 lines per mm. are more usual. They may be used either by depositing the specimen directly on to them, or as reference standards. That is, the magnification at which a microscope is being used on a specimen can be found by looking at a graticule placed in *exactly the same position* as the specimen. This caution is essential, since magnification depends directly on the distance of the specimen from the final microscope lens, as we shall see.

The micron is the ideal unit for measurements made with the light microscope, since the very best definition that can be obtained hardly exceeds 0·1 μ, even with ultra-violet light. The advent of the electron microscope, however, immediately extended our range of investigation by 100 times and through later developments by another ten or twenty times. A new micro-unit was needed and the obvious step was to divide the micron again by 1,000, to give the *millimicron* (in Germany, the 'nanometre'). It is usually abbreviated to 'mμ' and called the 'millimu'. It is equal to one millionth mm.: 1 mμ = 10^{-6} mm. It is a most convenient unit for discussing biological microstructures. The unit threads of nucleoprotein are about 2 mμ thick, the polio virus is about 30 mμ in diameter, the band

separation in collagen fibres is 64 mμ and the influenza virus is about 75–100 mμ across.

It happens, however, that all atoms and many molecules are still smaller than a millimicron, and so, quite independently of the needs of microscopists, a different unit of measurement had been set up by scientists concerned with studying the sizes of atoms and the patterns in which they arrange themselves. This unit was chosen so as to be smaller than the closest separation of atoms in crystals and compounds. It was named the *Ångström unit*, after a famous Swedish physicist, and defined as one hundred-millionth of a centimetre: $1\text{Å} = 10^{-8}$ cm. Hence it is one-tenth of a millimicron: 1 mμ = 10 Å. It is widely used by physicists, crystallographers and physical chemists, whereas biologists for the most part prefer the millimu. The wavelengths of X-ray and electron beams are in this range of dimension. From a 60 kilovolt tube the shortest emitted X-rays have wavelength 0·2 Å, from a 15 kV tube 0·8 Å. A 60 kV beam of electrons has wavelength 0·05 Å, a 15 kV beam 0·1 Å. Atomic sizes are rather larger. In rock salt the minimum distance between the sodium and chlorine atoms is 2·81 Å and in metallic gold the atomic lattice has a repeat distance of 4·05 Å along the cube edge. Similarly in non-crystalline compounds: the separation of hydrogen and oxygen in a molecule of water vapour is 0·96 Å and that between carbon and carbon in the backbone chain of a hydrocarbon molecule is 1·54 Å.

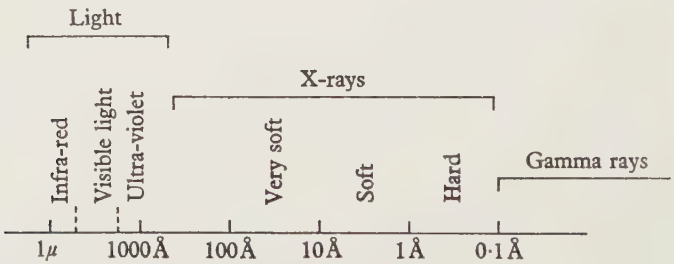

Fig. 1. Wavelength scale for the different types of radiation $\left(1 \text{ micron } (\mu) = \frac{1}{1000}\text{mm.}; \text{ 1 Ångström unit (Å)} = \frac{1}{10{,}000}\ \mu\right)$.

It is characteristic of a live and developing subject that there should be a certain amount of confusion and disagreement about the units of measurement. In this book we shall use the micron as unit in all matters connected with light microscopy, in spite of the fact that physicists are used to quoting the wavelengths of visible radiations in Ångström units. In electron and X-ray microscopy we shall follow current practice and use millimicrons for the dimensions of biological structures and micro-organisms, but Ångström units for those of atoms, molecules and crystal lattices. The chart in Fig. 1 will serve as a reminder of the relative values of these units and of the wavelengths of visible light, ultra-violet and X-radiation.

The Different Types of Microscope and their Limitations

The essential feature of a microscope is the ability to enlarge small objects, that is, to produce magnified images of them. The simplest means of doing so is a single biconvex lens, usually called a magnifying glass and only rarely by its proper name, the *simple microscope*. It is quite satisfactory for low magnifications, although it needs a well-designed (and expensive) lens to enlarge twenty times without blurring of the image at the edges, or noticeable colour effects. For observations at higher power the *compound microscope* has to be used. It has two stages of magnification, produced by the objective lens and the eyepiece respectively; each of these lenses in turn has to be made up of several component lenses, in order to make the image as true a likeness as possible of the object. The compound microscope, at best, has a maximum useful magnification of 1,000 times, written for short as 1,000 ×, of which the objective may provide 100 × and the eyepiece 10 ×. Beyond this limit we have to rely on the electron microscope, which uses quite different types of lenses. The most modern instruments work up to a direct magnification of 200,000 ×, but, since this electron image can be viewed with a low-powered light microscope, the working magnification may in fact be more than 1,000,000 ×. The various types of X-ray microscope

have so far not succeeded in doing better than the best light microscope, and usually operate at less than 1,000 × direct magnification.

Each of these different types of microscope is subject to certain definite limitations, partly set by basic physical facts (e.g. the wavelength of light) and partly by technical difficulties of

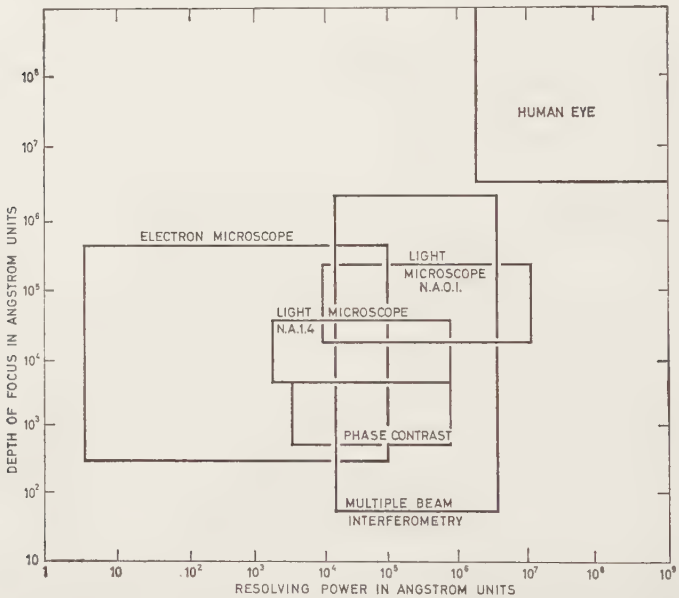

Fig. 2. The scope of different types of microscope and of the unaided eye, in terms of resolving power and depth of focus (1 mm = 10^7 Å; 1μ = 10^4 Å).

design and construction. For these reasons each method has a fairly well defined working range, as indicated diagrammatically in Fig. 2. We shall now enquire more closely into the limitations and usefulness of each of these tools of microscopy, giving at the same time some typical examples of what they can show us of Nature's various levels of detail. The type of biological specimen which occurs in each size range is shown in Fig. 3. Here, as is general practice nowadays, we shall always use 'magnification' in the linear sense as meaning the number of times by which a distance in the object is enlarged

in the image. In the early days of microscopy the magnification in area was often quoted, no doubt to impress—the areal magnification being the square of the linear magnification.

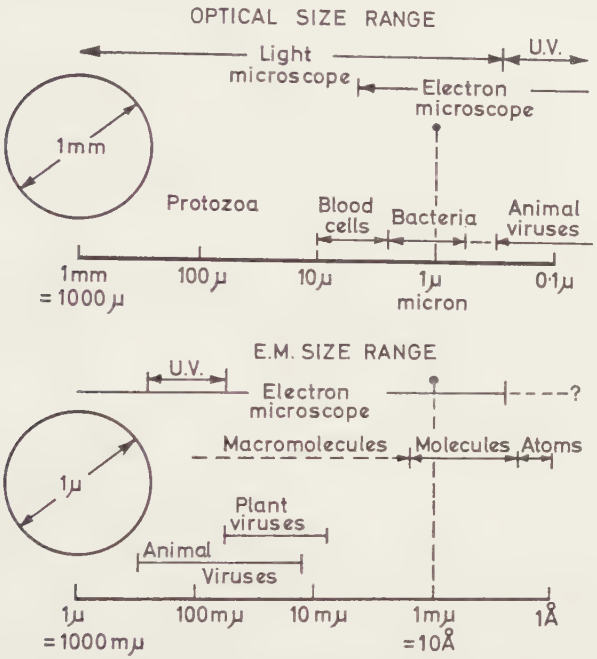

Fig. 3. Size ranges of the optical and electron microscopes, and the types of biological specimens occurring in them.

By 'magnifying power' or 'direct magnification' we mean the ratio of the linear size of an image to that of the object, as enlarged in the microscope. The image as finally printed may well have been further enlarged photographically, or, more rarely, reduced in size. For this reason the description of micrographs often contains both the 'original' and the 'final' magnifications.

(a) The Simple Microscope

A single biconvex lens (i.e. a lens thicker at the centre than at the edges, as in Fig. 4) will cause magnification if it is placed between an object and the eye. There are two positions of the

lens giving a sharp enlarged image, and we shall see in the next Chapter that it is best to hold the lens close to the eye and bring the object up towards it (p. 43). From one point of view the lens is simply a means of allowing an object to be brought up close to the eye without going out of focus; the nearer it is to the eye the bigger it looks, since the eye itself is a lens. A small hole in a sheet of metal or cardboard also allows the power of the eye to be increased without loss of clarity, and so a hole held close to the eye might be claimed as the simplest

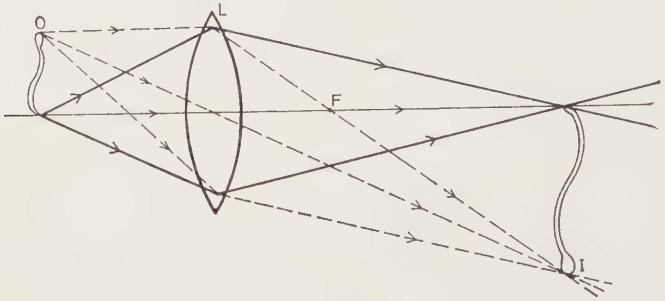

Fig. 4. Magnification of an object by a single lens; it becomes a 'simple' microscope when the lens is held close to the eye, as in Fig. 13.

form of microscope (see p. 45). A single lens is more useful because it has a magnifying power of its own, and also it provides a much wider field of view.

The magnifying power of a single lens is reciprocally related to its focal length—if the focal length is halved the magnifying power is doubled; hence the familiar observation that the 'fatter' a lens the stronger it is. At the same time more blurring is likely to occur: the image may not be in focus over the whole field of view at once, and parts may be coloured. By careful design of the lens, involving the use of several component lenses, these 'aberrations' can be corrected (p. 48). The best type of magnifying glass, as distinct from a low-powered reading glass, will consist of three lenses cemented together to give a combined focal length of 2·5 cm. and a magnifying power of 10 ×. A single lens of 20–25 × is about the useful limit of this type of construction, but not many of them are manufactured.

In many respects, however, the best form of lens is a complete sphere or ball, if used with the object in a particular position. This was known to the ancients, probably by observing the way in which a flask filled with water concentrates the sun's rays. The focal length of a sphere of glass is roughly equal to its radius, so that it has to be quite small for high magnifying power. It was the peculiar genius of the Dutch scientist, Leeuwenhoek, that he found means of making minute beads of glass of spherical shape and mounting them to form a simple microscope of very high power. He lived and worked at Delft from 1632–1723, and was the first scientist to look into the fine structure of plants, insects and animals. So remarkable were the details revealed by his work that the descriptions he sent to the Royal Society in London were not at first believed, until Robert Hooke was able to repeat and confirm them some time later.

One of Leeuwenhoek's simple instruments is shown in Plate 1A. It consists of a glass bead set in a metal plate to which is attached an adjustable pointer, on the end of which the specimen was fixed. The eye has to be placed close to the hole in the plate and the pointer carefully moved about until a clear magnified image of the specimen is seen. It needs delicate handling and very good lighting—preferably sunlight —to make observations at these high magnifications, and so Leeuwenhoek's work was rarely repeated in the 18th century and the excellence of his microscopic drawings was not surpassed even with the compound microscope until well into the 19th century. He made hundreds of these simple microscopes, of which only some ten survive. Careful measurements made by the Museum at Leiden, where most of these are preserved, have revealed that the strongest lens he made was only 1·5 mm. in diameter ($\frac{1}{16}$ in.) and has a focal length of about 1 mm. This corresponds to a magnifying power of 250 ×. With this type of lens, Leeuwenhoek was the first man to see bacteria, sperms (Plate 3A) and many fine details of plants and insects. These discoveries justify us in regarding him as the father of microscopy, even though he made little or no use of the microscope as we understand the instrument, in its compound form.

For many years it was thought that there was some secret process in the way Leeuwenhoek made the glass beads of his 'simple microscope', but attempts made in Holland to reproduce his work have recently shown that it can be done, given clever fingers and limitless patience. It may be, however, that he had some special way of polishing his lenses. With the best of them he was able to see microscopical details almost down to 1 μ—only ten times worse than the limit of the best modern compound microscope. Leeuwenhoek first observed sperms in 1677 and bacteria in 1683. It was as much as 150 years later before the compound microscope, already known before 1650, was improved sufficiently to beat his simple lens. For most scientific work during the 18th century, and even up to 1830, the simple microscope was preferred to the compound type. In 1829 we still find this opinion expressed, in an article in the *London Encyclopaedia*:

'Compound microscopes, though not so much to be depended upon for the purposes of discovery and philosophical investigation as single lenses, are still best adapted for recreation.'

In these days we use the single lens either as a reading-glass at very low power, or for viewing details of stamps, coins or insects up to a power of 10–15 × at most; for higher magnifications we always use the compound microscope. The reason is, of course, that although it may not be any more difficult to make 'bead' lenses than a modern microscope objective of high power, the combination of eye-piece and objective is much more versatile and much less exacting to use than the Leeuwenhoek microscope. The rise of the modern microscope, as we know it, dates from about 1830 and the main stages were completed before 1900.

(b) The Compound Microscope

It is not possible to name the discoverer of the compound microscope (Fig. 5). Since, in its earliest form, it is essentially a telescope used the wrong way round, the putting together of two lenses for that purpose must have been quickly followed by the discovery of their alternative use. It is certain that the telescope was in use in Holland by 1610 and in Italy, by

Galileo, soon afterwards. There is controversy as to whether the oldest instrument which seems to have been designed as a microscope, made by Janssen at Middelburg in Holland, is earlier than this. There is no doubt, however, that the Dutch

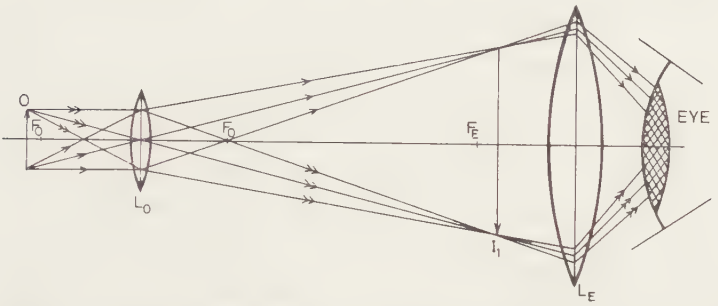

Fig. 5. Two-stage magnification in a compound microscope. The image I_1 formed by the first lens ('objective') is further enlarged by the second lens ('eye-piece').

Ambassador to the Court of James I, Cornelis Drebbel, had a compound microscope in his possession and knew how to use it when the Dutch scientist Constantin Huygens visited him in London in 1621. From this time the construction of microscopes was taken up very rapidly in Holland, Italy and a little later in England. In this country the great pioneer was Robert Hooke, who was responsible for scientific demonstrations at the Royal Society in its earliest days. In 1665 the first edition of his 'Micrographia' appeared, with wonderful drawings and descriptions of insects, crystals, feathers, and a host of other common objects which he had examined under his primitive compound microscope (Plate 2).

It was Hooke who was asked to verify Leeuwenhoek's report of the existence of 'little animals' in pepper-water, since the members of the Royal Society found it hard to believe. At first Hooke failed to find them, but when he followed more faithfully the instructions given in Leeuwenhoek's letter, he was able to confirm all the observations. As a result, the microscope was soon focused on practically every conceivable object in Nature. In particular, Nehemiah Grew carried out at Cambridge a systematic study of the botanical kingdom, his drawings of plant structure being among the classics of microscopy.

The microscope became an object of fashionable interest, no man of intellectual pretensions could be without one in his library, and makers of microscopes quickly grew up to supply the need. Many of these early instruments were works of art in their own right, their broad barrels of wood or cardboard carrying brightly painted designs. Their optical system, however, lacked the perfection of the exterior. To look through one of them is indeed to perceive an object 'in a glass, darkly', except at very low magnifications.

The reason for this poor performance lay in the fact that it was only possible at that time to make the simplest type of lens. As we shall see in Chapter 3, the different parts of such a lens have differing focal lengths, so that they form images at different positions ('spherical aberration'), and these focal lengths will also vary with the wavelength of the light coming from the object ('chromatic aberration'). With a small aperture, and light mainly of a single colour, the image can be quite good; but in white light and with an open aperture, so that all parts of the lens assist in forming the image, it may be very badly blurred.

The nature of these limitations was early realised, and a great deal of thought and experiment was devoted to finding a solution during the second half of the 18th century and early part of the 19th. Chromatic aberration was first corrected in telescopes, by making a lens of several components and using different types of glass for them. It was more difficult to make the small lenses needed for microscopes, and it was not until the early 1800's that the technical problem was satisfactorily solved, first in Holland, then in France. The main contribution from this country was made by the father of Lord Lister, the originator of anaesthetics, who made a chromatically corrected microscope about 1830. At the same time he showed the way to the reduction of spherical aberration by making the lens components of a particular shape and placing the object in a particular position.

Greatly improved compound microscopes were thus available by 1850, and the rest of the century is the story of the gradual perfection of lenses and of the mechanical parts of the instrument. For the first time it was possible to go beyond the observations made with the simple microscope, and there was

great activity in all branches of science in probing into the fine structure of Nature. Biologists in particular were busily laying bare the microscopical detail of living matter. Plate 3B shows how much more could be seen of the structure of spermatozoa than in Leeuwenhoek's day. In terms of the scale of sizes (Fig. 3) our powers of observation had been increased roughly tenfold, from a limit of a few microns to a few tenths of a micron. It made all the difference between seeing the details of a cell and merely the main outlines (Plate 4). With the invention of the ultra-violet microscope the observational limit was pushed still farther, to 0·1 μ or slightly less.

The compound light microscope became one of the most powerful tools for scientific research, and indeed is still often used as a symbol for it. But it soon was found in practice, as predicted theoretically, that the limit to its power of revealing detail was very definite and apparently insurmountable. This limit is set by the wave nature of light and can be directly related to its wavelength (p. 65). It is impossible to 'resolve' detail smaller than (roughly) one-third of the wavelength of the light being focused; for ultra-violet radiation, of 0·3 μ wavelength, the limit of 'resolving power' is thus about 0·1 μ. Although it was known that shorter wavelengths exist, there were (and still are) grave practical difficulties in focusing them.

The story of light microscopy in the present century, therefore, is largely one of extending the range of use of the instrument and making it easier to handle. The main advance has been in devising methods for examining objects, and especially living specimens, which are colourless and of much the same refractive index as their surroundings, and so are almost invisible in the ordinary microscope. As we shall see in Chapter 3, first the phase-contrast microscope and then the interference microscope opened up great new fields of investigation into living processes. Previously high-power microscopy had only been possible on thin sections of dead tissue; living cells could be observed only at comparatively low magnifications. Combined with the application of ciné and time-lapse techniques of photography, the new instruments are giving us a deeper insight into dynamic phenomena, such as that of cell division. Films of the action of antibiotics on

bacteria have been made and, in the inorganic domain, of the growth of crystals from solution.

More and more uses have been found for the light microscope, but its resolving power could not be extended. It was realised that X-rays are of much shorter wavelengths, and so ought to allow finer detail to be displayed, but it is almost impossible to focus them with lenses; the X-ray projection microscope, later devised, suffers from limitations of a different sort (see Chapter 5).

(c) The Electron Microscope

The way forward into the 'sub-microscopical' world was found when electron lenses were invented, about 1925, and it was realised that the very small size of the electron (whether we regard it as a particle or a wave-motion) should permit a very

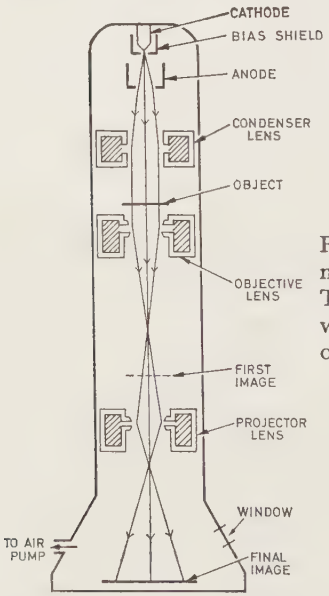

Fig. 6. Outline diagram of an electron microscope, with magnetic electron lenses. The image is formed on a fluorescent screen which is viewed through a window from outside the evacuated tube.

much greater resolving power than in the light microscope. Electrons can be focused (in a vacuum) by magnetic and electrostatic fields of special shape (Fig. 6), and it was found that the electrical engineers had solved many of the problems

when developing the cathode ray oscillograph. The first electron microscope was built in 1931–32 and the first pictures showing detail finer than that of the best light microscope were published in 1933.

The past thirty years has seen the very rapid development of the electron microscope itself and of its application in almost every branch of science and technology. Special methods of preparing specimens have had to be developed because, first, they have to be put into vacuum and, secondly, electron beams can penetrate only a very thin layer of matter, even after being accelerated with 100,000 volts. In any case it is desirable to have a limited thickness of specimen, as otherwise a vast amount of overlapping detail would appear in the image owing to the high resolving power and great depth of focus of the electron microscope. With the best modern instruments detail smaller than 1 millimicron (=10 Ångström units) can be resolved, so that even in a specimen as thin as 0·1 μ there might be 50 to 100 layers of structure of this fineness. Ways were soon found of cutting still thinner sections of tissues than are usual for light microscopy, and more recently techniques of thinning metal foils in a controlled manner have been devised. Some examples of the striking advances made with the electron microscope are given in Chapter 4. Plate 4c gives an indication of how much more information is provided about cell structure, and is typical of the contribution which electron microscopy is making in biological research. Metals also contain a quite remarkable wealth of detail (see Plates 11, 12 and 14), which can often be related to the large-scale properties of metals, such as ductility, hardness and brittleness. These results were largely unsuspected and have led to important advances in our understanding of metals and in their use in engineering.

The electron microscope is still in the early stages of development. In particular we have yet to learn how to correct the spherical and chromatic aberrations of our lenses. From the viewpoint of the optical designer, electron lenses are at the moment quite primitive. They give a resolving power some 300 times worse than the wavelength of the electrons would permit if the lenses were perfect. But in spite of falling so far

short of perfection, the electron microscope gives an improvement of almost 200 times over the limit of the ultra-violet microscope. This is because the electron wavelength is some 60,000 times shorter than that of ultra-violet light, about 0·05 Å (for 60,000 volt electrons) as compared with 3,000 Å (0·3 μ). Clearly the electron microscope has a long way to go, if only various practical difficulties can be overcome. At the moment its limit of performance is about 6 Å,* two or three times larger than the separation between individual atoms. In the meanwhile a very simple 'electron microscope', which dispenses altogether with lenses and relies on direct geometrical projection, has been able to make individual atoms visible in a metal (see Plate 20).

(d) Flying-spot Microscopes

In the microscope, as ordinarily understood, the image is formed all in one piece and is viewed (or photographed) as a whole. An alternative method is to build it up point by point, the different parts being formed and recorded in sequence instead of simultaneously. The object may be illuminated all over and scanned by the recording system point by point or line by line, as in a television camera, or the illumination may be limited to a fine pencil which is scanned across the object, so that it is illuminated sequentially whilst a recording system (photo-cell or counter) is permanently open before it.

Such a flying-spot or scanning microscope was first made for visible light, later with ultra-violet and now with an electron beam. It has the advantage of illuminating the object only for the time needed to get a picture, which is important when the specimen is radiation sensitive. A more general advantage is that the magnification and contrast are under electronic control, and the technique can indeed be used for enlarging and clearing up photographic negatives. A more important application, however, is for the counting and size-grading of particles, such as blood cells or dusts, with the aid of special electronic recording gear.

* By 1964 this limit had been reduced almost to 2 Å, on specially selected specimens such as a thin crystal of barium sulphate or a gold foil.

The resolving power of the method is clearly limited in the first place by the size of the flying-spot and secondarily by the characteristics of the recording and display system. It cannot do better than the ordinary type of microscope so far as definition is concerned. However, in its electron-beam form it has the advantage of being applicable to surface studies, which are very difficult to carry out in the usual electron microscope. The scanning electron microscope has now been developed into a practical instrument, and it is beginning to find uses in metallurgy and in the study of insulators and semiconductors, and especially of fibres. Quite recently it was realised that it can provide information about the chemical composition of a specimen. X-rays are emitted as the electron beam passes over it, and the elements present can be identified from the characteristic wavelengths they contribute to the spectrum; it is only necessary to direct an X-ray spectrometer at the surface being scanned. By careful comparison of the X-ray output with that given by standard samples of pure elements, the method can be developed into one of quantitative microanalysis. It permits point-by-point analysis of a specimen in a volume of less than 1 cubic micron (10^{-12} cc.), and an element can be detected when present only to the extent of 10^{-15} gr. in favourable circumstances.

The scanning electron probe microanalyser, as it has come to be called, is thus an instrument of many uses in metallurgy, mineralogy, and to a lesser extent biology and medicine. It can indeed be operated with a stationary electron probe, the specimen being moved under it. However, it proves to be advantageous to scan the spot over the specimen and use television techniques to display an image, by recording either the electrons reflected from the surface or the X-rays emitted. In either case an image at high magnification is formed and the instrument can be properly classed as a microscope. For this reason, and because of its value as a research tool, it is discussed in Chapter 6 along with the flying-spot optical microscope. It must be said at once that its resolving power as yet is far inferior to that of the normal type of electron microscope.

CHAPTER

2

THE EYE AND THE SIMPLE MICROSCOPE

THE SIMPLEST WAY of magnifying an object is to use a single lens of the convex ('bulging') type. If you hold it between your eye and an object, you will see an enlarged image of the object. It is in fact acting as a simple microscope, although it is more usual to speak of it as a magnifying glass. Practically all other microscopes, more complicated and higher powered as they may be, are built up on the focusing action of such a single lens. In this chapter we shall investigate how this focusing occurs, and how it depends on the material and curvature of the lens. We shall see that there are limitations to the maximum useful magnifying power of a single lens, but that some of them can be overcome by making it up from two or three semi-lenses. And, since it has to be used in conjunction with the eye, which itself is a lens, we must also consider the focusing action of the eye, both on its own account and in combination with the simple microscope. In the process it will become clear that there is a right and a wrong way of using a 'simple microscope' for looking at small objects.

The best way of learning about lenses is to use one; to look through it and see for yourself how an object appears in different positions. Hold an ordinary magnifying glass about half way between your eye and the lettering on this page, as you hold the book at reading distance. Bring up the book to the lens. The print will slowly increase in size at first, but upside-down, then quickly rush away into a blur; but after that it will equally quickly appear again the right way up and enlarged. Alternatively, if you keep the book fixed and move the lens from the eye towards it, the same sequence of events

occurs. In either case it is worth while to inspect carefully what you see near the position of maximum blurring. Holding the lens with a steady hand, you will see that a letter gets much distorted at the edge of the lens and also shows colour effects (unless you have a very good lens indeed). The lens is producing different magnifications at its outer zone and in its centre (spherical aberration), and slightly different images in each of the colours of white light (chromatic aberration).

Now hold the lens close to your eye and bring the book up towards it. There will only be a blur until the print is quite close, and then it will suddenly appear enlarged and upright—and with no trace of distortion or colour effects (unless you have a very bad lens indeed). You get rather less magnification than when the lens is held well away from the eye, but the image is much clearer; also the field of view is larger than when the lens is close to the book.

Finally, make three small holes in a piece of cardboard or thin wood (or better still a metal strip), about half an inch apart and 2 mm., 1 mm. and 0·5 mm. in diameter. Hold it up to the eye and look through each hole in turn at a printed page. Bring the print slowly up towards the eye and find the closest position at which you can still read it easily. The smaller the hole, the closer you can read the print and the larger it appears. The hole, in fact, has much the same effect as a lens held close to the eye, except that the image is less bright and the field of view smaller, owing to the small diameter of the hole. With the smallest hole, also, you will get a certain amount of blurring of the print even in the best position, due to an effect known as diffraction of light at the edges of the hole.

These observations show that a lens can be used in a number of different ways, which result in differing optical appearances; sometimes the image is upright, sometimes inverted. In some conditions the lens gives an image suffering from certain imperfections, the spherical and chromatic aberrations, but when it is held close to the eye these defects are much less noticeable. Also, there is something about the eye itself which enables a simple hole to act in much the same way as a lens. We have to explain all these observations in terms of the way in which light is bent ('refracted') in passing through a

lens. Although the lens is one of the first topics discussed in school Physics, we shall begin at the very beginning, so as to be sure that no essential point is missed.

Refraction of Light; Prisms and Lenses

A lens is actually very similar to a prism of varying angle. When a beam of light passes through a prism, i.e. a block of glass with two opposite sides inclined at an angle, it will be appreciably changed from its original direction owing to the refraction which occurs at each surface. It is deviated through a definite angle, and this angle of deviation, D, depends on the angle of the prism, A, and on the material of which it is made. Since by focusing we mean the ability to bring all parts of a wide beam together at a single point, this requires an increasing amount of deviation for parts of the beam farther away from the axis. A set of sections of prisms of increasing 'roof' angle, piled up on each other, will have exactly this effect (Fig. 7).

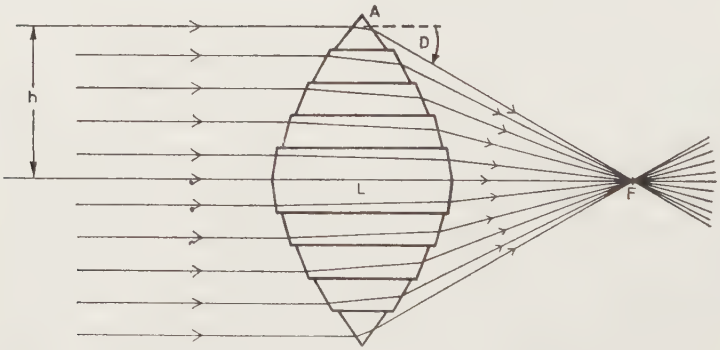

Fig. 7. A pile of prism sections has a focusing effect similar to a lens.

With the help of a little geometry and trigonometry the nature of this focusing action can be explored in more detail, leading us to relations for the focal length and the magnification. It is worth while trying to follow the steps in the argument, as it helps to make clear why lenses are made as they are, and why they suffer from certain limitations.

The amount by which a ray of light is deviated when it passes into any transparent material, such as glass, depends on

the refractive index of the material, for which the symbol μ is used ('mu'). The higher the value of μ the more the ray is refracted. Typical values are: crown glass 1·52, flint glass 1·65, diamond 2·42, perspex 1·49, teflon 1·35, water 1·33, benzene 1·50.

If now we have a thin prism of angle A, as in Fig. 8, the beam going through it will be refracted twice: at the first surface

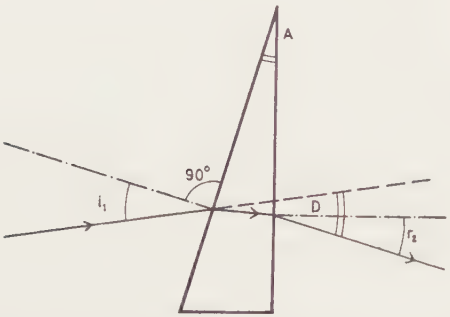

Fig. 8. A ray of light entering a thin prism is refracted slightly at each surface, and so deviated towards the base by the angle D. i_1 = angle of incidence at first face and r_2 = angle of refraction at second face, both being measured from the perpendicular.

towards the perpendicular, and at the second surface away from the perpendicular, since it is emerging into air here. The total effect on the beam is measured by the angle of deviation D, the angle between the incident and emergent beams. The value of D will depend on the prism angle A as well as on the refractive index μ, the relation between these three quantities being

$$D = (\mu - 1)A,$$

so long as the angle A is not too large, i.e. the prism is thin.

This simple relation between angle of deviation and prism angle is the key to the focusing action of a lens. To bring all parts of a parallel incident beam to a common focus, at a point F, we have only to set up a pile of prism sections in which the angle between opposite faces increases in direct proportion to the distance (h) from the axis, as in Fig. 7. The distance LF

is then called the *focal length*, f; i.e. it is the distance from the lens to the image when the object is at a very great distance, effectively at 'infinity'. So far as focusing in the plane of the diagram is concerned, a convex lens is thus equivalent to a large number of consecutive prism sections. A lens which would focus in all planes through the axis could be made by cutting circular grooves in a sheet of plastic, with bevel angle increasing with radius, as shown in Fig. 9. Such 'flat lenses' have in fact been made, now that suitable plastics are available.

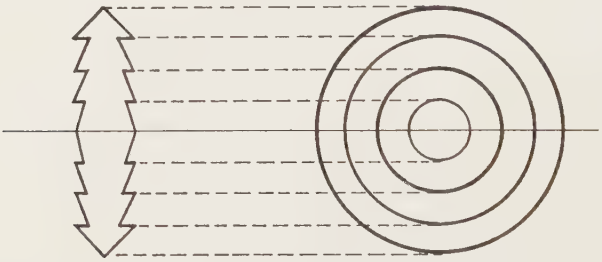

Fig. 9. A flat sheet of perspex, in which circular grooves of increasing bevel angle are cut, will act as a lens.

The ordinary sort of lens, with faces ground and polished to spherical curvature, happens to be an easier practical way of producing such a focusing action. It can be regarded as made up of two separate lenses, each with one face flat and the other curved ('plano-convex'), but not necessarily identical, as in Fig. 10. Such a single semi-lens will be defined by its radius of curvature R_1 and its edge-angle, A_1, which corresponds to the angle of the top prism of the equivalent pile (Fig. 7). So long as the lens is not too thick, the equation for D given above can then be reduced to

$$1/f_1 = (\mu - 1) \cdot 1/R_1$$

thus relating the focal length to the curvature of the lens. A similar relation holds for the second semi-lens, of radius of curvature R_2 and of focal length f_2 when used alone. On putting the two semi-lenses together, the total deviation will be the sum of that due to each separately and, again for a thin lens, the combined focal length f is given by

$$1/f = 1/f_1 + 1/f_2 = (\mu - 1)(1/R_1 + 1/R_2)$$

So we arrive at the basic 'lens equation', relating the focal length of a lens to its radii of curvature and refractive index. It shows that to make a strong lens, of short focal length, we need a material of high refractive index and surfaces of small radius of curvature. Whatever material we choose the first bracket will still be less than unity, unless we are rich enough to afford a gem stone! A flint glass lens ($\mu = 1{\cdot}65$) has a focal length about half that of a water lens ($\mu = 1{\cdot}33$), for a given shape. Freedom of choice is really confined to the radii of curvature, and the limit here is set by the mechanical difficulty of making highly curved surfaces. Supposing the lens to be of glass of $\mu = 1{\cdot}5$, and to be equi-convex so that $R_1 = R_2 = R$, we can reduce our focal length equation to*

$$1/f \fallingdotseq 1/R$$

so that the focal length is roughly equal to the radius of curvature. It is important to write it as an approximate equality, since our discussion is strictly valid for thin lenses only, and we are now talking of strong lenses, which necessarily are thick.

Another point to bear in mind is that the lens has been assumed to be in air, the refractive index of which is taken as unity. When immersed in another medium, such as water, of refractive index μ', the first bracket in our expression for f becomes $(\mu - \mu')/\mu'$ instead of $\mu - 1$. So the power of the lens is reduced. If it is placed in a medium of refractive index equal to its own, $1/f$ is zero and it no longer acts as a lens; how could it, since no refraction can occur now at its surfaces? If placed in a medium of still greater refractive index, such as carbon disulphide ($\mu = 1{\cdot}67$), a biconvex lens would be reversed in power and act as a diverging lens.

We see then, that a glass lens to be of 1cm. focal length (in air) must have radii of curvature of about 1 cm. Its maximum diameter will be 2 cm., when it is made as a sphere. As a hemisphere, i.e. a plano-convex lens, which is more convenient for a microscope objective with the specimen near the flat face, the radius would have to be 5 mm. and the diameter 1 cm. for $f = 1$ cm. We can now see why Leeuwenhoek used beads of glass for the lens of his simple microscope. A bead of radius

* The sign $\fallingdotseq$ means 'approximately equals'.

1 mm. would have a focal length of about 1 mm. By the same token, its diameter would be only 2 mm. and its field of view of the specimen correspondingly restricted. What magnification it will give is a more complicated question since the answer involves the observer's eye, which has its own focusing action. We shall shortly take a look at the eye, and then at the eye looking through a lens. But first of all, what do we mean by magnification?

Magnification

A lens is normally used to form an enlarged image of an object, and the magnification is simply the number of times the image is larger than the object. To be more precise, we should say that the linear magnification is the height of the image, measured at right angles to the axis, divided by the height of the object (Fig. 10): $M = I/O$. The magnification in area will be the square of this: $M_A = M^2 = I^2/O^2$. Later on we shall be talking about angular magnification and also axial magnification—the ratio of image thickness to object thickness. For the present we may suppose the object to be one-dimensional, an arrow perpendicular to the axis.

The geometrical construction of Fig. 10 shows how the image can be found when the object position and the focal length of the lens are known. A ray parallel to the axis from

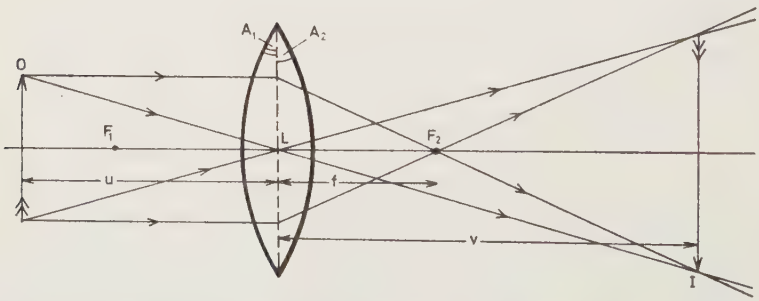

Fig. 10. Imaging with a single lens, showing focal length f, object distance u, and image distance v. F_1 and F_2 are the front and back focal points; A_1 and A_2 are the 'prism angles' at the circumference of the two half-lenses of which the actual lens can be regarded as composed.

the head of the arrow must pass through the back focal point, F_2, by definition. A second ray, directed towards the centre of the lens, will pass through with negligible deviation if the lens is thin. The point of intersection of these two rays locates the head of the image *I*, and a perfect lens would cause all other rays from *O* to pass through the same image point. From similar triangles about the lens centre it follows that

$$M = I/O = v/u,$$

the magnification being given by the ratio of image distance v to object distance u. This is of practical importance, since it allows us to find the magnification from a knowledge of these two distances, instead of having actually to measure the size of the image formed of a given object. We may even avoid having to measure the image distance, once u and f are given. It is not difficult to prove from Fig. 10 the well-known formula:

$$1/f = 1/u + 1/v.$$

So, by eliminating u, or v, we can write the magnification as

$$M = f/(u-f) = (v-f)/f = \frac{v}{f} - 1.$$

Two points are worth noting. First, the signs in these expressions may look wrong if you have been used to a different convention for positive and negative directions from that assumed here: that 'real' distances (to locatable objects, images and centres of curvature) are positive, 'virtual' distances negative. So long as you stick to a given convention there is no need to worry, the numerical result will come out the same in the end. Secondly, these expressions are true only for thin weak lenses, but analogous relations can be obtained for strong ones. As might be expected, distances are then not measured from the lens itself. It is notionally split into two refracting elements, each concentrated at appropriate positions (the principal planes) so as to give the same combined effect as the thick lens itself, and distances are measured from them. This approach is even more important for electron lenses, where practically all lenses are extended along the axis and must be regarded as thick.

We now have to examine more closely the way in which magnification changes with object distance. It follows at once from the expressions for the magnification that it will increase rapidly as u decreases. But it is also useful to look at the geometrical relations, as in Fig. 10. The object can be imagined as travelling towards the lens on guide rails, the rays from its head and foot parallel to the axis. The focused direction of these, through F_2, is fixed for a lens of given focal length and does not change as O is moved. The other image-fixing rays, through the centre of the lens, are in effect pivoted at L and open out as O approaches the lens. The points of intersection of these rays, after refraction, with those through F_2 will run off rapidly to the right and occur at increasing distance from the lens. The image distance v increases and its size increases in proportion; in fact, it runs away to infinity as O approaches F_1, the front focus of the lens. In terms of Fig. 10, this occurs when the rays pivoted about L become parallel to those through F_2 and so can only meet them at infinity.

What happens when O is moved inside the front focus and the emergent rays are diverging from each other? They now only meet if produced backwards, along the dotted lines of Fig. 11, to form a 'virtual' image at I'. The position of this

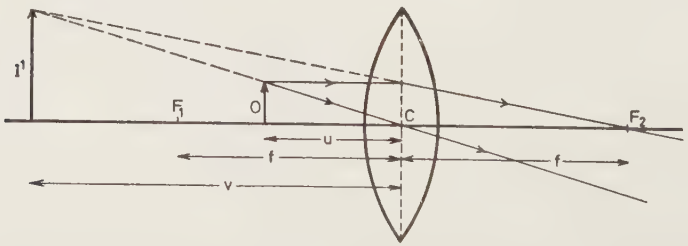

Fig. 11. Imaging with a single lens when the object is inside the front focus; a 'virtual image' I' is formed where the emergent rays appear to diverge from.

image still obeys the lens expression $1/f = 1/u + 1/v$, provided that we now label its distance negative $(-v)$. In a sense it has a 'real' existence. If we place a second biconvex lens close to L, I' can be treated as the object for this lens, which will produce an image of it according to the lens relationships, as

if it were a real image at I'. Indeed, this is just what the eye does when you hold a magnifying glass up close to it.

The Eye as a Lens

Fig. 12 is a section through a human eye (other living creatures such as fish or insects have very different eyes), and shows that its lens is highly curved and is really made up of three parts, the cornea, C, the aqueous humour A, and the

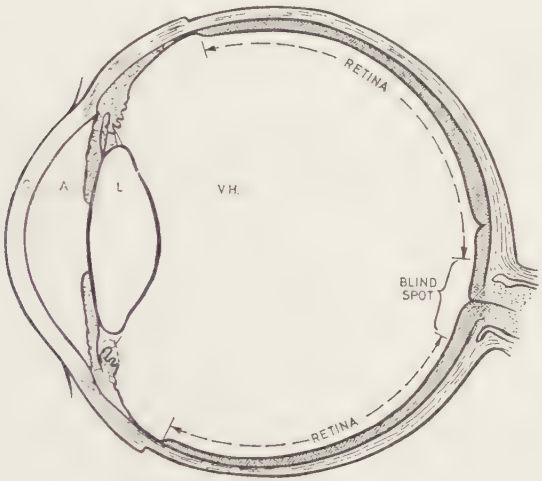

Fig. 12. The Human Eye. C = cornea; A = aqueous humour; L = crystalline lens; $V.H.$ = vitreous humour.

crystalline lens L. It is a thick lens, with two components, but it focuses light in just the same way as the simple lens considered above. However, it has two special features: it works at fixed image distance, and has a variable focal length. Indeed, the one is a necessary consequence of the other. The image formed by the eye falls on the sensitive retina at the back of the eye-socket. Hence image distance v is practically constant, a very different state of affairs from that considered in Fig. 10, where v was able to range widely as the object was moved in front of a lens of given focal length. For the eye, the

only way of coping with the situation is to change its focal length for different object distances. If one of the quantities is fixed, the basic relation $1/f = 1/u + 1/v$ can be satisfied only if both the other two change in the right proportion. In the usual lens experiment (Fig. 10), v changes with u; in the eye, f changes with u.

This change in focal length is called the accommodating power of the eye, and is brought about by automatic action of muscles which squeeze the crystalline lens and change its radii of curvature. For distant objects, the focal length is a maximum and consequently so also are the radii; the eye is relaxed. As the object is brought up to the eye, focal length and radii must decrease. There is clearly a limit to the accommodation possible, when the eye reaches its maximum curvature and minimum radii. This limit corresponds to the 'near-point', which you can measure for yourself by finding the nearest approach to your eye at which this print can still be read. It may be as low as 8–10 cm. at age fifteen, but gets longer with age, usually being about 50 cm. at age fifty. The range over which the eye's focal length can be varied is found as follows. The length of the eye socket is about 20 mm., so $f = 2$ cm. for the relaxed eye, when the object is 'at infinity'. If the near point is 20 cm. ($= u$), the value of f must become 1·82 cm. to keep $v = 2$ cm. (check by the lens formula!). The optician expresses the same facts in terms of the 'power' of the eye lens, common-sensibly measured as $1/f$ (with f in metres) in units of dioptres.

Note that the linear magnification of the image on the retina will vary correspondingly as the eye accommodates. $M = v/u$, so that M is very small for distant objects and reaches a maximum at the near point, where it will be $2/20 = 1/10$; in other words, it is always fractional. More surprisingly, we see that the image on the retina must be inverted, since u is always greater than f (as in Fig. 10). Does this mean that we are always seeing the world upside-down? Indeed it does—although there are some people who still say that it is indeed an upside-down world, and that we are living on the inner surface of a hollow sphere, with gravity acting outwards! However, it is rather difficult to fit the stars and the solar

system into such a system, apart from the fact that we do not get to Australia by taking off vertically from the ground.

To convince oneself of the reality of image inversion in the eye, a simple experiment can be made with a pin and a strip of metal or cardboard. Make a hole about 1 mm. in diameter in the strip and hold it at arm's length in front of a bright light. Now slowly pass a pin up across the eye and close to it. You will see a blurred rod moving *down*, in the opposite direction. Doubting Thomases can convince themselves that this proves 'mental inversion' by repeating the experiment on the bench with a big pin in front of a large lens, and a screen just behind it, with an illuminated pin-hole at the other end of the bench; the shadow on the screen moves *up*. So the brain turns the optical image upside-down. Indeed, no other conclusion is possible since the eye contains no second lens for re-erecting the inverted image. Why, then, do we interpret the world the right way up nevertheless? The answer seems to be that we learn by trial and error, often no doubt painful, in our infancy. We find that we can only reach things or get to places on the assumption that we are looking at everything upside-down. This process of experimentation can be seen going on in the efforts of babies to reach objects put in front of them. It is reliably reported that a man who puts on a pair of prismatic spectacles, which re-inverts the world, soon learns to react normally to it, and, when he leaves off the spectacles, sees everything upside-down with his unaided eyes. For the philosophically minded this is a valuable argument to use against those idealists who would have us believe that the world exists only in the mind of the individual observer!

The Eye plus a Simple Microscope

We must now enquire how it is that a lens used near to the eye produces a considerably enlarged image, and how the magnification changes with the position of the object. Try it. When the object is well away from the eye only a blur is visible, but if it is brought up quite close to the lens a clear enlarged and upright image is seen. The object is inside the front focus of the lens and a virtual image is formed (as in Fig. 11), which the

eye lens is able to focus on to the retina. For the eye to be able to do so, the virtual image must obviously lie at or outside its near-point. In practice the observer subconsciously adjusts the object position so as to get the virtual image at about the distance at which he would normally hold a book for reading —the distance of distinct vision, D. The situation as regards ray paths and image formation is then as shown in Fig. 13.

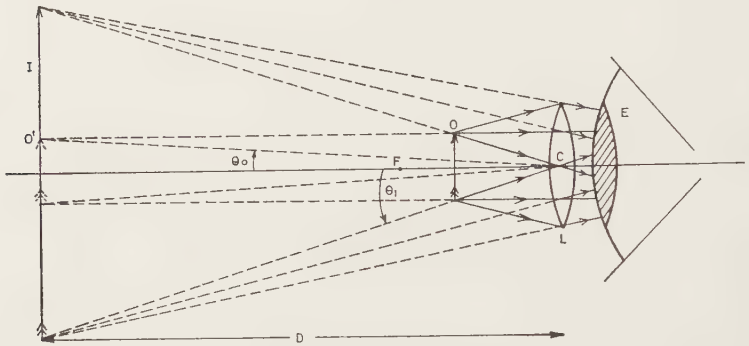

Fig. 13. Single lens used as a simple microscope, close to the eye. The virtual image I is formed at the distance of distinct vision D, where the object would normally be placed for viewing without a lens. θ_1 is the angular half-height of the image and θ_0 is the angular half-height of the object at distance D.

The magnification produced by the lens is given by the expression which we derived earlier, but with the image distance now equal to D:

$$M = v/f - 1 = -(D/f + 1)$$

D is negative because it refers to a virtual distance. As the distance of distinct vision is about 25 cm. for most people, this is taken as the standard value of D for reckoning magnifying powers. So a lens of $f = 5$ cm. will give a magnification of six times when used in this way, or if $f = 2$ cm. then $M = 13{\cdot}5\times$. A Leeuwenhoek lens of $f = 1$ mm. would give a magnification of 251 times.

What would happen if the object is placed actually at the front focus of the lens? The virtual image should then be 'at infinity', since the rays forming it are parallel; if v is infinite then the linear magnification ought to be infinite. That

this is not so can be checked at once by moving an object about in front of a lens held close to the eye. Over the range of u for which a clear image is visible there is hardly any variation in size of image, M is almost constant. To resolve this apparent contradiction we have to go over to the idea of *angular magnification*, M_a, which is defined as the ratio of the angle θ_1 subtended by the image at the eye to the angle θ_0 which the object would subtend if placed at the distance of distinct vision. In this sense the magnification produced by the lens, when used in combination with the eye, is the number of times larger the object appears than when viewed directly at the normal distance D; this is sometimes called the 'effective', instead of the angular, magnification.

When the image is formed at D, as in Fig. 13, the angular magnification is equal to that defined in the usual way, in terms of linear size:

$$M_a = \theta_1/\theta_0 = I/O = M = -(D/f + 1)$$

as above. When the object is moved out to the front focus, the image moves away to infinity so that v and I become indeterminate, but the angle subtended by the imaging rays hardly changes from its previous value. In this position the angular magnification is

$$\begin{aligned} M_a &= (O/f)/(O/D) \\ &= -D/f \end{aligned}$$

So the magnification is decreased by unity when we put the object at F instead of at the position giving the image at D. For a lens of $f = 5$ cm, M will be 5 instead of 6 times. Hence our experimental observation is explained. Remember that this is so only because the lens is being used in conjunction with another lens, that of the eye; no image would be formed on a screen placed in the position of the eye in Fig. 13.

A Hole as a Lens

From the angular point of view, a lens is simply a device which allows us to look at an object much nearer to the eye than usual; and the object seems enlarged in the ratio of its

two distances. A small hole in a strip acts in much the same way—not because it focuses light, of course, but because it limits the area of the eye used and thus increases its depth of focus. The action is just the same as 'stopping down' the lens of a camera to bring both near and far objects into focus in the same picture. The key point is this: that when we say 'into focus' we really mean 'not blurred by a visible amount'. More precisely, the grain size of the developed photographic film sets the limit; so long as the rays from an object point intersect the plane of the film in a disc smaller than this, the object can be considered to be 'in focus'. In other words, the problem arises from the need to record, on a particular surface, images which are truly focused on a number of different planes, according to the distances of the corresponding objects.

The position is as set out in Fig. 14. Two object points, O_1 and O_2, produce images I_1 and I_2. A 'disc of least confusion', C, is formed where the respective rays cross in a plane between them, and the size of this disc obviously depends on the height

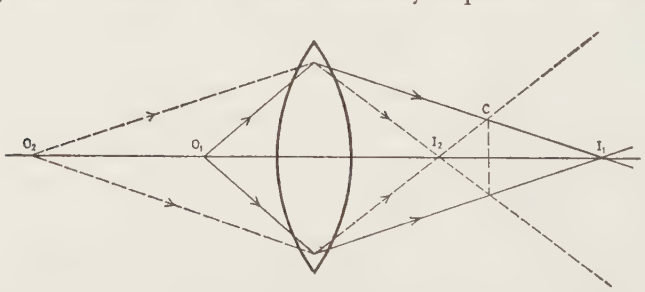

Fig. 14. Rays from all object points on the axis between O_1 and O_2 will pass through the disc of least confusion C on the way to their respective image points between I_1 and I_2. O_1O_2 is the depth of focus for a given size of C.

in the lens at which the imaging rays are focused. If, on the other hand, we fix the permissible size of the disc of confusion, then with a wide aperture the object points have to be closer together to keep the ray intersections in the right places. As the lens aperture is reduced, or 'stopped down', the outer object point can move farther and farther away and still give the same limiting size of disc. It is usual to say that the 'depth of focus' of the lens is thereby increased. It is more exact to

say that the depth of focus in the image is increased, and the depth of field in object space. Since the position of the image changes very little when distant objects are moved, it is primarily the closest distance of clear imaging which is affected by varying the lens aperture.

A hole in front of the eye acts in essentially the same way. By limiting the width of the eye lens taking part in image formation, it increases the depth of field and in particular brings the near point closer to the eye. An object at the near point then seems enlarged in the ratio D/u', where u' is its new distance from the eye. The distance of the near point is in fact directly related to the aperture of the eye. If R_L is the

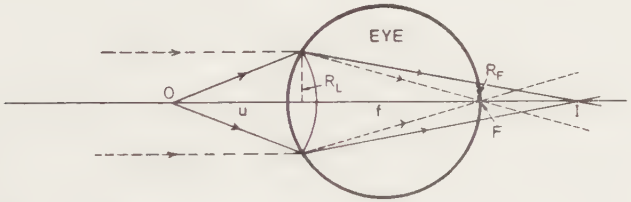

Fig. 15. Near-point of the eye: R_F is the disc of confusion for all object points between infinity and the near-point O. For given size of R_F, O can be nearer to the eye-lens if a smaller area of the lens is used—i.e. if an aperture reduces R_L.

radius of the pupil of the eye (see Fig. 15) and R_F the limiting size of the disc of confusion on the retina, at focal distance f from the eye (considered as a thin lens) then

$$u = f.R_L/R_F$$

So if we stop down the lens pupil to one-half, we also reduce the distance of the near point u to half its normal value; and since $M = D/u$, the effective magnification is doubled. Taking the diameter of the pupil as 3 mm. and the near point as 18 cm. for a normal eye, we shall reduce the latter to 6 cm. by looking through a hole of diameter 1 mm. and gain an effective magnification of 3 times. However, as mentioned earlier, there is a limit to the possibilities of the 'magnifying hole', since below about 0·5 mm. diameter not enough light gets through and also the image suffers from diffraction blur. Still, in the absence

of a proper lens, a hole makes a good substitute; if you are short-sighted, you can make shift with a pair of holes at the right separation, if you happen to break or lose your spectacles.

This discussion has made the large assumption, of course, that the eye can be treated as a thin lens when in fact it is a very thick lens. But the simple conclusion about the proportionality of near-point and aperture holds good pretty well for the way an actual eye behaves. Another consequence is that your near-point moves away in dim illumination, as the pupil opens. The same general argument can be used to calculate the depth of focus of a camera lens. But then we are likely to be interested in a narrower range of focus, with the far point not at infinity as assumed here for the eye.

Limitations of the Simple Microscope

In this argument we have considered a disc of confusion formed by the crossing of rays from *different* object points, supposing the lens itself to be perfect. In fact, lenses do not form a point image of a *single* point object; however small the object, the image point is always a disc of finite size due to the various defects from which a lens can suffer. The chief of these defects are the chromatic and spherical aberrations, which arise from lack of perfection of lens form and so are called geometrical aberrations, and the diffraction effect, which would occur even in a perfect lens because of the wave nature of light.

Chromatic Aberration

In your experiments with a simple lens, you will have observed coloured fringes around the image in some circumstances. They arise because white light is a mixture of all colours, and a lens sorts them out. In fact, this is bound to happen, since the refractive index of glass or any other material depends on the wavelength of the ray refracted. Probably many readers will have been worried by the idea of a lens as a pile of prism sections, knowing that a prism produces a spectrum from white light. It is indeed this prismatic effect that gives coloured edges to the image. Red and blue light, at the two ends of the spectrum and differing appreciably in

wavelength, are focused to different points by a given lens (Fig. 16). A single clear image will only occur if we illuminate

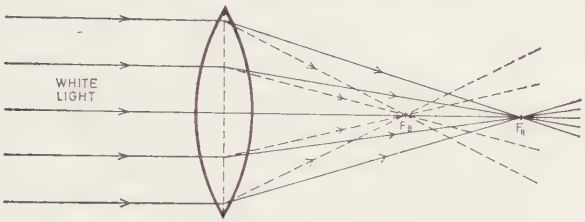

Fig. 16. Chromatic aberration of a lens: F_R is the focus for red light (full lines) and F_B that for blue light (dotted lines), other colours being focused between these points.

the object with light of a single wavelength, so-called monochromatic light. All our previous diagrams and lens equations are only strictly true for such a condition. Values given for refractive index and focal length should properly be accompanied by a statement of the wavelength for which they are valid. We shall write the refractive indices of a given material for red and blue light as μ_R and μ_B, respectively, and the mean value as μ_M. For most types of glass their difference is less than 1 per cent; the difference in f, for a lens of given curvature, is between 1 per cent and 2 per cent, depending as it does on $(\mu - 1)$.

This chromatic effect was known from the earliest days of lens making and was discussed by Newton, amongst others. It was not until well after his time that a way of correcting it was found. The solution depends on the fact that the dispersions produced by prisms made of different glasses, but having the same angle, are not proportional to the deviations. The ratio of the dispersion, measured by $(\mu_B - \mu_R)$, to the deviation D, which is proportional to $(\mu_M - 1)$, can vary widely from one glass to another. So we may have two lenses of exactly the same focal length, but one with much more chromatic aberration than the other. Conversely, they may give the same dispersion whilst having quite different focal lengths. In particular, a concave lens may be made which will have equal and opposite dispersion to that of a convex lens made of another sort of glass, and yet the latter will be much stronger,

so that the combination will have a positive focusing action. This principle was used from about 1757 for correcting the chromatic aberration of telescope lenses, the two components being of flint and crown glass respectively. A patent for it was granted in 1758 to John Dollond, one of the famous early instrument makers, but it seems certain that others had known of it earlier. As is not unusual with important inventions, there was much argument about who really was first. It is possible that several lens makers came on the principle quite independently, either by cogitation or by trial and error. Certainly the practical use of it came first and a proper theory was worked out only some years later—again a sequence of events frequent in the progress of Science.

Various combinations of materials for chromatic correction are possible but flint glass and crown glass are most used, because they differ widely in dispersive power. Crown glass, a sodium-calcium silicate of moderate density (2·5), was so called from the shape of the blown glass when flattened out. Flint glass is a potassium-lead silicate of higher density (3·6), and took its name from the use of crushed flints in the original method of manufacture. The refractive index of crown glass for red light is 1·517 and for blue light 1·523; for flint glass the values are 1·650 and 1·664 respectively. The dispersive power, given by the difference in μ for the two colours divided by $(\mu_M - 1)$, is thus 0·0115 for crown and 0·0213 for flint glass, i.e. flint has about twice the dispersive power of crown. So a prism of flint glass need have only half the angle of a crown prism to give equal dispersion, and correspondingly will give only half the deviation. So if we invert one of the prisms with respect to the other (Fig. 17*a*), the total effect will be about half the deviation of the crown prism alone and no net dispersion—blue and red rays will be brought to the same focus. Going over to complete lenses, a combination of a crown glass convex lens with a flint glass concave lens of half the power (Fig. 17*b*) will form an 'achromatically corrected doublet' for these two wavelengths.

All good magnifying glasses are now made in this way. But it was only many years after Dollond that it became technically possible to make them in the small size suitable for microscopes, in contrast to the big lenses of telescopes. By 1824 Chevallier

was making achromatic microscope objectives in France, and in 1829 J. J. Lister, the father of Lord Lister the discoverer of antisepsis, made a better one in England. Much later, in 1883, the firm of Zeiss produced the 'apochromat', corrected

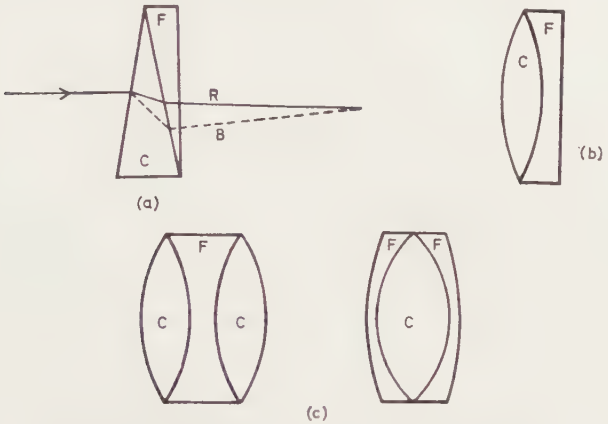

Fig. 17. Achromatic doublets: (*a*) a combination of a crown glass prism (*C*) with a thinner flint glass prism (*F*) brings both red and blue light to the same focus, with a weak combined focusing action; (*b*) and (*c*) are different ways of combining a concave flint lens with one or more convex crown lenses to form a chromatically corrected magnifier.

for a third wavelength in the middle of the spectrum by adding a third component, made of fluorite. These triplets are very expensive, on account of the difficulty of obtaining large enough samples of clear fluorite, and are only used for the best microscope objectives. In these lenses the components are in contact, or nearly so. By separating the components an extra degree of freedom can be obtained, and it is in this way that eyepieces are chromatically corrected.

The triplet magnifier, 10 × or 15 ×, which you may be lucky enough to possess, is composed of two crown and one flint lenses (Fig. 17*c*), the crown always being the positive, biconvex component. When held in the proper way, close to the eye, it is difficult to detect any colour in the image except perhaps at the very edge of the field, and it gives a flat field image. A single uncorrected lens does nearly as well over the centre of

its field. Although its focal length varies appreciably with wavelength, giving rise to chromatic difference in magnification, the separate images are formed close behind each other. With the lens very close to the eye, as in Fig. 13, these are seen largely along the principal rays *IC* through the centre of the lens. Thus they overlap and fuse in the eye to give the impression of white light. When the lens is held at a greater distance, chromatic effects are much more pronounced because all the field is used and because the images are more widely separated.

The eye itself shows chromatic aberration, of course. Its lens, being largely water, has moderate dispersive power, but normally the pupil is small and only the centre of the lens is effective. In the dark, with the pupil dilated, colours can produce noticeable confusion. Many people find difficulty in seeing clearly two patterns projected side by side on a screen, one with blue and the other with red light. Or a distant purple light seen in the dark will appear as a blue dot with a red ring around it. In general, however, the eye is a remarkably good lens from the point of view of both chromatic and spherical aberrations.

Spherical Aberration

As mentioned earlier, spherical aberration arises from the outer zones of a lens having a different power from its centre. In a spherical biconvex lens the focal length is shorter for the outer zones than for the paraxial zone, as in Fig. 18. The magnitude of the aberration is usually measured, not by this

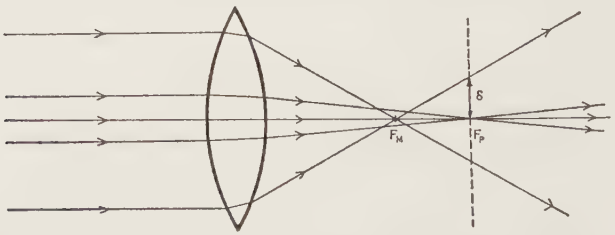

Fig. 18. Spherical Aberration: light passing through the marginal zones of a convex lens is focused to F_M, closer to the lens than the focus F_P for light passing into the lens close to the axis. δ = transverse aberration.

focal shift, but by the radius δ of the disc of confusion formed by the outer rays intersecting the true, paraxial image plane, as shown; this is the 'transverse aberration'. The simplest cure is obviously to stop down the aperture of the lens, but what is to be done for lenses which must necessarily be of wide aperture, such as microscope objectives? The solution is found in 'bending' the lens: changing its shape, without altering its power, until minimum aberration is found.

The line to follow is clear from the nature of the over-focusing, since this occurs in the outer lens zones where deviation is greatest: reduce the deviation as much as possible at each refraction. With a single lens, the best arrangement will be that in which the marginal ray passes symmetrically through the 'prism edge' of the lens, the deviation being shared equally at the two surfaces. As this is the position of minimum deviation for a prism, the marginal focus is as far as possible from the lens (for given power), and the spherical aberration a minimum. Calculation shows that, for glass of $\mu = 1{\cdot}50$, the ratio of the radii of curvature must be 1 to 6 for this condition to be satisfied. In fact, a plano-convex lens has only slightly greater aberration and is much easier to make, so it is usually preferred. But it must be used the right way round—if placed with its plane face to the incident light the aberration is about four times worse. For a flint glass lens, of $\mu = 1{\cdot}66$, plano-convex is the best form exactly.

The spherical aberration can be still further reduced by dividing the total deviation among as many surfaces as possible, i.e. by splitting the lens into several components. The transverse aberration δ is approximately proportional to the cube of the angle of deviation, D^3. So for two refractions, each of $D/2$, the aberration is reduced to about $1/4$ $(= 1/8 + 1/8)$ of what it is for a single refraction of D. By splitting the lens into two weaker plano-convex lenses we spread the action over four refractions, each of $D/4$, and the aberration is reduced to very small proportions. However the lenses have to be separated by a finite distance, x. Calculation shows the best solution to be when the two components have focal lengths (f_1, f_2) in the ratio of the heights at which the marginal ray is incident on them, and when $x = f_1 - f_2$. Fortunately these requirements

can be closely approximated when chromatically correcting a lens, so that your triplet magnifying glass is also spherically corrected to a high degree. In any case, as Newton first showed, the chromatic error vastly exceeds the spherical in the simple uncorrected lens.

So far we have assumed parallel light to be entering the lens, or, since ray paths are reversible, the object, O, to be so close to the focus that an effectively parallel beam emerges, corresponding to high magnification. Are the conclusions similar if the object is elsewhere? In fact the spherical aberration varies greatly with object position, and may be either smaller or larger than when O is at the principal focus. In particular there is one position for which it is zero, giving the so-called 'aplanatic points', if (and only if) the object is in effective optical contact with the lens. Consider a sphere of glass of radius R, as in Fig. 19. It can be shown that an object point

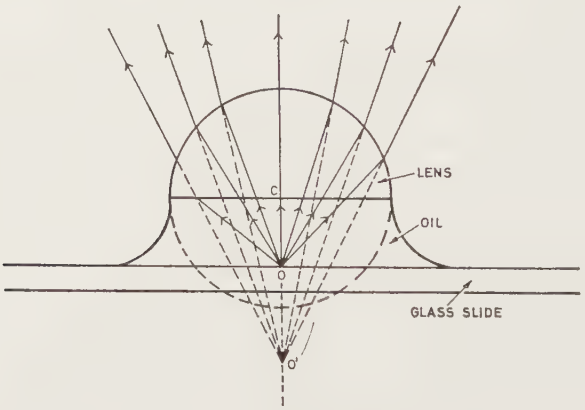

Fig. 19. Immersion Objective and Aplanatic Points: rays from the point O all emerge from the lens as if they came from the point O'.

at O, at a distance $(2/3)\ R$ from the centre, will be perfectly imaged at O', at a distance $(3/2)R$ from C along the same diameter. Being a virtual image, at least one further lens is needed for practical utilisation of this spherically corrected lens. The object could be got into the proper position by slicing off a section of the sphere, but this would leave a lens of awkward shape to make. It is simpler to use a hemisphere and

to fill the space between *O* and the flat face with an oil of refractive index equal to 1·5, or whatever the value may be for the glass used (see Fig. 19). Such a lens is known as an immersion objective and proves to have other valuable properties besides zero spherical aberration, as we shall see in the next Chapter. Consequently, it is the choice for all high-power microscope objectives, with cedar-wood oil as immersion medium.

It was shown by Lister in 1830 that two similar points exist for a thin achromatic doublet, and these are usually called after him the 'Lister points'. Although they give zero spherical aberration, they differ from true aplanatic points in that another aberration, coma, is not zero. To discuss coma and the remaining types of aberration would take us too far afield. They begin to make their presence apparent when the major afflictions of a lens, the chromatic and spherical aberrations, have been corrected, but they must be coped with by the optical designer when image definition of the highest quality is required. In the 200 years since John Dollond, a combination of mathematical analysis and technical skill has brought the art of lens design to a level at which the geometrical aberrations are so well corrected that the performance of a lens is limited by the wavelength of light itself.

It may be asked whether a lot of this trouble could not have been avoided by a different approach. Since these aberrations arise from using refracting surfaces of spherical curvature, would it not have been more fruitful to calculate the shape which will focus perfectly all rays whatever their distance from the axis? This problem was in fact solved by Descartes and others in the 18th century, only to be replaced by the even more difficult problem of making the necessary non-spherical surfaces and, equally important, of knowing when we have reached the right shape. Spherical surfaces are both mechanically much easier to grind and polish, and simpler to test for trueness. So, although parabolic and other aspherical surfaces are often made for mirrors, their use for lenses is rarely attempted. It is simpler to stick to surfaces of constant radius of curvature, and to use combinations of several of them in series to offset the inherent aberrations of a single lens.

Conclusion

We see that the major aberrations of glass lenses can be corrected by sufficient thought and skill, and images of high perfection can be obtained. Used close to the eye, a simple microscope can give quite high magnification, but this is reciprocally related to the focal length and radius of curvature. So a single lens of high power is inevitably small in size. It is therefore difficult to make and correct, and awkward to use; in particular it has a very small field of view. For these reasons pocket magnifiers are rarely made of power greater than about 20, corresponding to a focal length of 1 cm., although for lack of any alternative for high magnification, Leeuwenhoek made lenses as small as 2 mm. in diameter and of magnifying power approaching 250 times. Since his heyday we have had 300 years in which to develop a much more practicable instrument, capable of ten times this magnification, in the form of the compound microscope.

CHAPTER

3

THE COMPOUND MICROSCOPE AND ITS LIMITATIONS

The compound microscope is at the same time one of the simplest and one of the most complex scientific instruments. In essence it is only a simple microscope with an extra lens added to give higher magnification. In its finished form it is in fact a masterpiece of optical design, both the objective and the eye-piece being multi-component lenses of carefully chosen materials and calculated shape. By these means the aberrations—coma, astigmatism, distortion and curvature of the field, as well as chromatic and spherical aberration—are all corrected to the highest degree. The compound microscope is indeed one of the most perfect of instruments, in that very little more can be done to improve its performance. It was brought to this pitch of perfection between 1830 and 1880, and not without reason is it so frequently used as a symbol of scientific research. Since that time improvements have been mainly concerned with making it more convenient to use aud adapting it to new needs, such as time-lapse (slow speed ciné-photography) and high or low temperature work. Its wavelength range has also been advanced well into the ultra-violet region of the spectrum.

It follows that there is not a great deal new to describe, beyond what is contained in school 'A'-level text-books, about the compound microscope as ordinarily understood. It is important, however, to see why the 'perfection' of the instrument is relative and limited: it is as perfect as we can hope to make it, within definite limits set by Nature and connected with the wavelength of light. We shall investigate the phenomenon of diffraction and arrive at a quantitative measure

of image definition, the 'resolving power', as determined by it. As this relates the wavelength of the illumination to the size of the finest detail that can be seen in an image, improvement can only come from using a radiation of still shorter wavelength than visible light. The ultra-violet part of the spectrum can be used up to a point, but the technical difficulties are severe and we end up with a mirror system in a vacuum chamber. X-rays, still shorter in wavelength, are very attractive for microscopy, but pose difficulties of a different sort due to their penetration through lenses and mirrors, as we shall see in Chapter 5. An electron beam provided an easier way of getting higher resolution, once methods of focusing electrons were discovered (Chapter 4).

There is, however, quite a different direction in which the compound microscope has been developed, in the past twenty years or so, and that is for the observation of transparent specimens. Since most living matter in the natural state comes under this heading, the value of the new microscopes will be apparent. The visibility of objects in the ordinary microscope depends on the amount by which they absorb light passing through them—it is a question of reduction in the intensity or amplitude of the light waves, and we speak of 'amplitude contrast' as the important factor. But the velocity of light in the specimen will usually also be less than in air; this is what gives rise to a different refractive index. So the waves passing through it will be slowed down with respect to those travelling a path of equal length in air. A peak in one wave-train will fall slightly behind that in the other, or, in more exact terms, a phase-difference has been introduced between them. By clever, but fairly simple, optical tricks the two sets of waves can be brought together again and the difference in phase made visible as a difference in amplitude. We need, therefore, a means of splitting the original light beam into two paths, a means of reuniting them and some method of shifting the phase of the reference beam with respect to that passing through the object, so as to convert phase to amplitude effects and incidentally to allow contrast to be controlled. Since we start with a single beam, the final beams are 'coherent', that is to say, optical interference takes place between them.

Understandably the finished instrument is usually called an 'interference microscope', but this is really a misnomer since interference also plays an important part in the normal microscope, as we shall see. It would be more correct to call all these new instruments 'phase-contrast microscopes', but it happens that a particular type (with no control over the phase difference) has come to have this name.

Until these new instruments were produced it was only possible to see the fine details of living specimens by staining them with dyes, so as to make them absorb light of a particular colour, and this meant killing them ('fixing' them, in the usual phrase). First the phase-contrast microscope, and later the more complicated interference microscope, opened up the possibility of examining living organisms and tissue-cultures at a magnification high enough to reveal cell details. By fixing a camera and clockwork mechanism to the microscope, micrographs could be taken at regular intervals of time. Projected at normal cinema speed, such films then showed the processes of cell division much more clearly than was possible before. It is not too much to say that the interference microscope has produced a revolution in our knowledge of the developing cell, not least because it permits quantitative measurements to be made of the refractive index and thickness of cell components. We shall describe later the principles of these new instruments and the sort of information which they provide.

The Compound Microscope: Principles and Construction

The basic lay-out of the compound microscope of normal (or 'amplitude') type is very simple, as shown in Fig. 20. Essentially it is a means of providing the simple microscope with an enlarged image to look at, instead of the object itself, so that a high final magnification is obtained. The first lens, or objective L_0, is of high power and the object O is placed just outside its front focal point, so that a greatly enlarged first image is formed a long way behind it, at I_1. The eye-lens,

with the eye near to it as in the simple microscope, is arranged so that I_1 falls just inside its front focus, resulting in the formation of a further enlarged virtual image I_2. The total magnification is the product of that due to the two lenses individually. The distance L_0I_1 has come to be fixed at 16 cm. by agreement among microscope designers, but whether this was

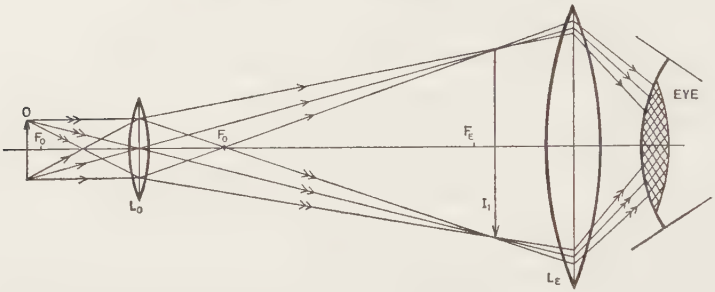

Fig. 20. The Compound Microscope: the eye and the eye-piece lens L_E form a simple microscope for further enlarging the image I_1 formed by the objective lens L_0.

done to keep the barrel short, or because it produces the final image at about the same distance as in the simple microscope, it is not clear. So if the object lens is of focal length 2 mm., $u_1 = 2$ mm. approximately and $M_1 = 160/2 = 80$. With a $\times$ 10 eye-piece an overall magnification of 800 is obtained. As before, we can also consider this as an angular magnification: the ratio of the angle subtended at the eye by the final image to that which the tiny object would subtend if looked at directly. We shall explain later on why we can use as objective a lens of high power which would be largely useless as a simple microscope.

Obviously the magnification can be varied over a very wide range by changing the objective and eye-piece. Most modern microscopes have three or four interchangeable objectives on a rotating turret (Plate 1B), but recently a 'zoom' objective has been made with which an appreciable range of magnification is available simply by turning a ring which changes the separation of its component lenses. Two new problems arise, however, in the compound microscope as compared with the simple lens: in regard to illumination and to field of view. A magnification

of 1,000 times will enlarge the object by one million times in *area*. For the image to be bright enough for the eye to see clearly, the illumination on the object must be greatly intensified—if not by a million times at least by several thousand times. The early microscopists such as Hooke used sunlight, focused on to the specimen by means of a round flask of water. Nowadays we use powerful point-filament or small arc lamps and a condensing lens. For ultra-violet microscopy, when magnifications as high as 3,000 may be demanded, only a high-powered mercury lamp will suffice.

The difficulty of width of field is evident from Fig. 21. Only a narrow cone of light from the objective can be collected by the

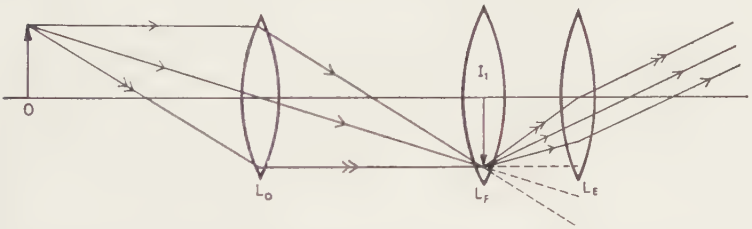

Fig. 21. Field Lens: L_F bends into the eye-piece (L_E) rays from the objective (L_0) which otherwise would miss the eye-piece, so that a larger area of the object becomes visible.

eye-piece. The best we can do is to place a convex lens, as shown, to bend some of the outer rays into the eye-piece. If it is placed in the plane of the primary image, it acts without effect on the magnification. This method of using a field lens was known before the time of Hooke, being first mentioned by Huyghens in 1654. That it offers only a partial solution is clear from the facts of the situation. The field of view of the objective lens will be of the same order as its focal length, say 2 mm. If it is producing a primary magnification of 800 ×, the width of the first image field will be 16 cm., falling close to the front focus of the eye-piece. Any practicable field lens can only cover a small part of this area, probably 2·5 cm. at most. So the field of view in the eye-piece is usually equivalent only to a small fraction of a millimetre in the object. Since the specimen stage is normally movable with micrometer

screws in two directions at right angles, this is not a serious restriction in practice.

Correction of Aberrations

As already mentioned, the compound microscope is corrected to a high degree for all the aberrations described in connection with the simple lens, and some others. We must now enquire how this can be done. In the eye-piece it is not a difficult problem, as there is plenty of room for manœuvre. But in the objective, where short focal length is demanded, only a very complicated set of lenses will give adequate correction. Requirements are much more stringent here, since every defect is magnified by the full magnification of the microscope; in the eye-piece a defect can be ten times more severe without being noticeable. Or, looking at the situation from a different viewpoint, the imaging pencils entering the eye-piece are much narrower than those in the objective, where we are always trying to use the widest possible aperture.

The Objective Lens

The main component of the objective is a hemispherical lens, with flat face to the specimen, in order to take advantage of the aplanatic property mentioned on page 54. But, so long as the specimen is in air, some spherical aberration remains, and, since the front lens is in one piece, it has considerable chromatic aberration. Both these defects are corrected with two doublets set at carefully calculated positions behind it; they have to be 'over-corrected' in themselves, to cope with the front lens aberrations. When highest performance is needed, an immersion objective is used (cf. Fig. 19), the space between specimen and front face of the hemisphere being filled with oil of refractive index equal to that of the glass of which this lens is made. It is then desirable to fit a 'meniscus' lens immediately behind it, so as to reduce the divergence of the cone of light passing up the microscope tube. The complete lens train is thus rather complicated (Fig. 22), and is known as an immersion achromat. Even so, it only corrects spherical

aberration for one wavelength and chromatic aberration for two wavelengths. To correct the former defect at two wavelengths and the latter at three requires a system of ten lenses, in the form of the apochromatic objective.

Having got his lens right for these aberrations, the optical designer is likely to find that it only gives a perfect image for a

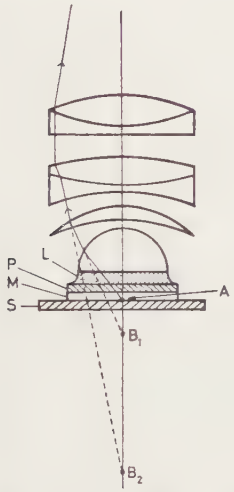

Fig. 22. Component lenses in an immersion achromat for the compound microscope. L = immersion liquid; M = specimen section; P = cover glass; S = glass slide. (*From* A Text-book on Light *by A. W. Barton; Longmans, London.*)

very small region of the object close to the axis. Points off the axis will be slightly distorted, by an amount depending on their distance away from it and also on the aperture of the lens. The total effect on the image can be analysed into a number of component aberrations, named after the characteristic shape they give to what should be a point image. Coma, for instance, draws out a tail from it, like a comet; astigmatism forms it into a horizontal line on one side of true focus and into a vertical line on the other side. We cannot go further into the problem here, which has occupied the lens designer for over a hundred years. It fortunately turns out that the worst of these minor aberrations, coma, can be corrected by the same means as are used for spherical aberration. Astigmatism and distortion also go together, and can largely be corrected by proper choice of the refractive indexes of the two components of the doubtlets. Curvature of the field presents difficulties, i.e. the imaging of a flat object as a curved surface. This is more

important in the eye-piece than in the objective, since (as already mentioned) only the centre of the primary image formed by the latter is further magnified and this can be made reasonably flat.

The Eye-piece

It is desirable to use a compound eye-piece, of two lenses, instead of the simple magnifying glass for viewing the image produced by the microscope objective. The reason is two-fold: first, to catch as wide an area as possible of this primary image—i.e. the first lens of the eye-piece is the field lens mentioned earlier (see Fig. 21)—and secondly, to correct the chromatic aberration which afflicts the outer parts of the field of a single lens, even when used correctly as a simple microscope close to the eye. This effect is connected with spherical aberration. Different types of eye-piece have been evolved to meet these needs, but they all rely on two basic principles. First, by spreading the total deviation of the imaging pencils over four refractions, the effect of spherical aberration can be greatly reduced; and, secondly, the use of two separated lenses allows chromatic aberration to be corrected. For, in the first lens the red wavelengths will be refracted less than the blue, and so they strike the second lens higher and will thus be refracted more strongly here. By setting the two lenses at the right distance apart, chromatic correction is obtained. The two most used eye-pieces, the Ramsden and the Huyghens, differ in the relative strength of the two component lenses and in the way they are arranged. As so often in the art of lens correction the designer has to make a compromise about the residual aberrations; Ramsden made one choice and Huyghens a different one.

The Illuminating System

The specimen needs to be very brightly illuminated, if we are to be able to see easily an image at high magnification, as we saw earlier. For this purpose a lens system is fixed below the microscope stage so as to focus light from a powerful lamp on to

the specimen. The lamp is conveniently placed on the bench near the microscope, or built into its base, and a plane mirror is used to reflect the light beam into the condenser lens, as it is called. In the best microscopes the condenser lens is also a multi-component system, primarily to correct chromatic aberration. It is important to have it positioned properly, with the specimen at its back focus, and so a moderately fine adjustment is provided for moving it up and down. At high magnification, when every error upsets fine definition in the image, it must also be accurately centred on the axis of the microscope tube, with centring screws.

Such a condenser can only be used, of course, for transparent objects. When surface detail has to be examined, of a metal say, the illumination must be directed on to the specimen from above. This may be done sufficiently well for low-power observation by means of a lamp and lens set up at one side of the microscope tube. In microscopes made specially for metallography a lamp is fitted into the side of the tube and a half-silvered mirror or prism system is used to send light down through the objective, which thus acts as its own condenser.

Diffraction of Light and its Effect on the Microscope Image

If the only limitation on the sharpness of the image in a microscope were the residual aberrations of the lenses, then we might hope to see finer and finer detail in the specimen by putting up the total magnification higher and higher. We define the maximum useful magnification as that value which will enlarge the smallest image detail up to a size that can be seen by the unaided eye, i.e. equal to or larger than its resolving power. The smallest detail of interest may be equated with the disc of least confusion in the image, after the optical designer has done his best, denoted by δ. The resolving power of the eye in normal conditions of viewing can be taken as 0·2 mm. Hence the maximum useful magnification of a microscope, M_x is given by

$$M_x = 0{\cdot}2/\delta$$

if we express δ in mm. If δ has the value of 1 micron, as it may well have in a student's microscope, M_x is 200 $\times$. Any higher magnification will simply show the same amount of detail on a larger scale; blurring will be visible, and we shall say that the image is 'over-enlarged'. If δ is 0·2 micron, not far off what it is in the best microscopes, then M_x is 1,000 $\times$.

We have to ask why it is that the optical designers do not go still further with lens correction, so as to raise the maximum useful magnification to 2,000 or 3,000 times, even if this meant having to put in an extra lens to make it a three-stage enlarging system. It is already getting difficult to meet the requirements of a microscope designed to magnify 1,000 or 1,500 times, and the technical optics expert is probably glad not to be asked to go even twice as far again. The essential reason we do not ask him to try, is that the nature of light itself makes it futile to solve the problem. The illumination we direct on to the object is a wave-motion, the waves of which are effectively disturbed only by structural details in the specimen which are larger than a certain size. Unless some change is imposed on the waves, we cannot expect to detect that these details were in their path; if no signal is sent out, none can be received. The basic interaction which determines visibility in this sense is called 'diffraction', which results in a deviation of the waves as they pass an object. In this respect it has a superficial similarity to refraction, but differs in being explicable only in terms of wave-motion. We find, on careful investigation, that it is impossible to detect any object detail much smaller than about half the wavelength of the illumination, as indeed can be deduced from simple observations on water waves.

A 'ripple tank' is an apparatus for making experiments of this sort. It consists of a shallow container, with a flat bottom in which a glass window is usually fitted so that ripples on the water surface can be projected on to a large screen by a lantern underneath. The ripples are continuously generated by a vibrating plate at one side of the tank, connected to an electrical oscillator. When a large metal cylinder is placed upright in the water, the ripples bend out and round it, and the disturbance can be seen in the wave pattern for a long way beyond it. If we were prevented from seeing the cylinder, and

simply had a photograph of the water surface in the rear half of the tank, we could readily deduce that some object was present in the front half. By experiments with objects of known size, we could find rules for deducing the size of an unknown object. But if we decreased the diameter of the cylinder below a certain size, we should have difficulty in detecting any disturbance due to it in the ripple pattern behind it. An object in the path of a wave must be above a certain size for its presence to be 'visible'. It is not easy to arrive at a simple expression for this limit of detectability of a single object, relating its size to the wavelength of the ripples. It is much easier to do so for a pair of objects, since they produce a characteristic pattern in the ripples which we can measure and interpret. For this reason the 'resolving power' of an imaging system is always discussed in terms of a pair of particles, and is defined as the minimum separation at which we can detect that two particles are present, not one.

Reducing the problem to its simplest form, we have straight rows of ripples (a 'plane wave') impinging on two small discs, as in Fig. 23. The ripples curl around the discs and spread out

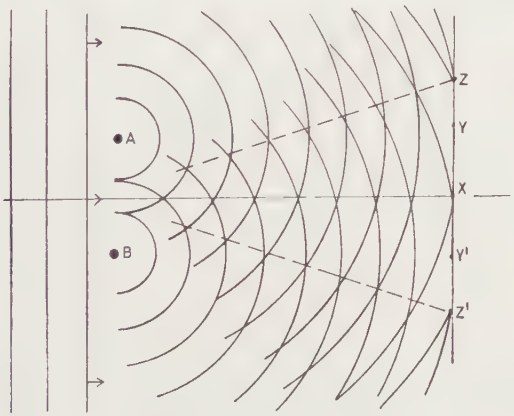

Fig. 23. Interference of two wave-motions: the straight wave-fronts arriving from the left are diffracted at the disc-objects *A* and *B*, and the diffracted waves radiating from these centres reins force (*X*, *Z*, *Z'*) or neutralize (*Y*, *Y'*) each other in a regular sequence at a distant detecting plane. The full lines represent crests of the waves.

in rings beyond. To a good approximation we can regard each disc-object as generating circular waves which are superimposed on the original plane wave. When these two circular wave-sets reach the far boundary of the tank, they will form a regular pattern of disturbance along it. On the axis drawn between the discs, the two wave-sets will always be 'in phase'; that is to say, a crest of each will arrive at the same instant, followed by two troughs, and again two crests, and so on. If we imagine a wave-detector at the point X, it will receive a strong signal. A little way off axis, at Y or Y', however, the distance from A will be just that much greater than from B as to result in a trough from B arriving with a crest from A. The two wave-sets will cancel each other completely, and this will be so for all states of the waves; a detector at Y or Y' would receive no signal. In between X and Y the signal strength will fall off regularly, then rise again beyond Y to a new maximum at Z where a crest from B arrives exactly one wavelength behind one from A, and so reinforces it. This succession of maxima and minima repeats regularly along the boundary. The waves from A and B are said to 'interfere' with each other, giving a pattern the exact scale of which will depend on where we place the detector-boundary, but which will always have the same form. Maxima occur along clearly defined directions radiating out from AB, and minima between these directions. This pattern is called a 'diffraction pattern', the objects A and B being said to 'diffract' the original wave system. In theory we could deduce the size and separation of these objects from sufficiently careful measurements of the details of the pattern.

Resolving Power

We can transfer these ideas directly to the case of the optical microscope. The ripples are now light waves illuminating a pair of black dots close together and in front of the objective lens, as in Fig. 24—though in fact the dots would be much smaller than can be depicted on this scale. The light will be diffracted by the dots, and in certain directions the diffracted wavelets will reinforce each other; in between these directions they will cancel each other, giving a minimum of light. The

beams of maximum light are termed the first, second, third, etc. orders of diffraction; the beam along the axis, where there is also reinforcement, is the zero order beam. The more of these beams that enter the objective lens, and contribute to forming the image, the more exactly does the image depict the original objects. But it might be guessed that the image will be good enough to show two separate objects so long as the objective

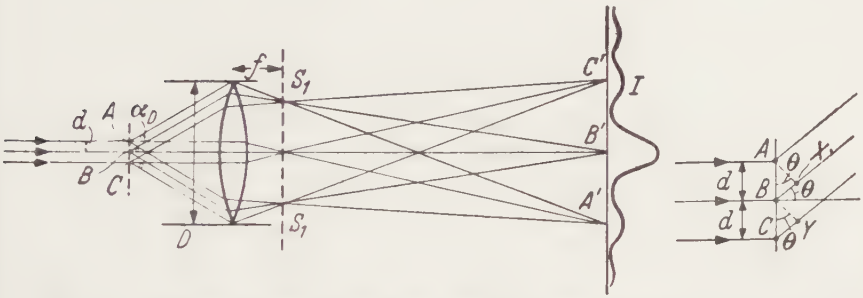

Fig. 24. Diffraction and resolving power in an objective lens. The incident light is diffracted by the row of object-dots and the first direction (at angle θ) in which reinforcement occurs is determined by the separation of the dots d and the wavelength of the light λ. α_D, the angular aperture of the lens, sets a limit on the resolving power. (*From* Practical Electron Microscopy *by V. E. Cosslett; Butterworth, London.*

collects at least the first order beam (as shown), and careful experiment proves that this is so. From this observation we can go on to obtain a relation between the optical properties of the objective, the wavelength of the illumination and the minimum detectable separation of two points—the resolving power of that objective.

The essential point is that in the first-order diffracted beam the wavelets from the two objects A and B are out of step by just one wavelength, and so are really in step again. That is, the path from B to the objective is longer than that from A by λ, using this Greek letter (lambda) for wavelength. If AX is the perpendicular from A on to the wave path (or ray) from B, cutting it at X (see inset on Fig. 24), the path difference is BX, and it follows that

$$\sin \theta = BX/AB = \lambda/d$$

Here d is the separation of the two points and θ is the angle

made with the axis by the first diffracted beam. So the closer together the points are brought, the wider will be the angle θ needed to give a path difference of one wavelength.

The minimum detectable separation will be reached when the first-order beam just enters the objective lens at its rim; that is, when $\theta = \alpha_D$, where $2\alpha_D$ is the angle subtended by the lens as seen from the object (see Fig. 24). This minimum value of the separation is called the *resolving power* of the objective and is denoted by δ, the Greek letter delta. The angle α_D (alpha) is known as the angular aperture and sin α_D as the *numerical aperture* (N.A.) of the lens. Notice that the *relative aperture*, often quoted for camera and telescope lenses, is different again, being the ratio of the focal length to the lens diameter; for instance, in an f/2·5 lens the focal length is 2·5 times the lens diameter.

So we get for the resolving limit of a microscope objective:

$$\lambda/\delta = \sin\theta = \sin\alpha_D = (\text{N.A.})$$

giving

$$\delta = \lambda/(\text{N.A.})$$

In other words, the resolving power will be better (i.e. smaller) the shorter the wavelength of the illuminating light and the larger the numerical aperture of the lens. This is the reason for making the diameter of a microscope objective as large, and the focal length as short, as possible. In the best modern lenses the N.A. may be as much as 0·95, and by filling the space between object and lens with immersion oil the value can be raised to 1·4 or even 1·5. The reason for this is that the wavelength is shorter (by $1/\mu$) in a denser optical medium because light travels slower in it. Hence the numerical aperture is properly written as

$$\text{N.A.} = \mu \sin\alpha_D$$

The resolving power can be improved by using converging or tilted illumination. It is enough for the diffracted beam on one side of the axis only to get into the lens aperture, the axial beam itself just getting in at its opposite rim. The absolute limit of resolving power is thus written as:

$$\delta = 0{\cdot}5\lambda/\mu \sin\alpha_D$$

Since the maximum possible value of $\mu \sin\alpha_D$ is about 1·5, the

limit of resolving power will be about one-third of the wavelength of the illuminating beam. In the middle of the visible spectrum the wavelength of light is about 0·5 μ and so we cannot expect to resolve object details, much smaller than about 0·2 μ, even in the best optical microscope. With a dry objective of N.A. = 0·6, the resolving power is about equal to the wavelength of the light used, and this will be achieved only if we have set up the microscope properly. (See remarks on using the compound microscope, on p. 82.)

The extent to which the detail seen in a test object, in this case a diatom from well water, improves as the lens aperture is increased can be seen from the series of micrographs in Plate 5. We shall come back later to discuss what improvement is obtainable by reducing the wavelength of the illumination.

Depth of Focus

But first it is interesting to consider the depth of focus of a microscope objective, as it proves to be very different from that of the unaided eye, which we discussed earlier (p. 46). As Fig. 25 shows, there will be a certain region along the axis over which the bundle of rays forming the image will be closer

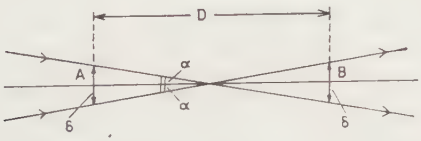

Fig. 25. Depth of focus of a lens: if the minimum distance which can be resolved by the lens is δ, then all points between A and B will be in focus in the image.

together than the distance δ we termed the resolving power. An object of width δ, or two objects this distance apart, can be anywhere in this region and still give the same image 'fuzz' in the viewer's eye. The depth D within which this is so,

measured along the axis, is determined by the angle of divergence of the imaging rays ($=2\alpha$) and the resolved separation δ:

$$D = \frac{\delta}{\sin \alpha}$$

Since sin α is nearly equal to one in a high-powered objective, the depth of focus is not much larger than its resolving power. So at high magnification we can see only a thin section of the object in sharp focus at any given setting of the fine-focus knob. By turning the latter we can sweep this 'optical section' through the specimen, bringing successive layers into focus. The rest of the specimen causes only a general diffusion of the illuminating light, but this can begin to obscure fine detail if the specimen is too thick. For best results it is found that its thickness should not be more than about ten times the resolving power. This is the reason why so much care has to be spent on cutting thin sections of biological material for microscopy, by means of a microtome. If detail as small as 0·2 μ is to be resolved, the section should be only 1 to 2 μ thick.

The Ultra-violet Microscope

Since the resolving power depends directly on the wavelength of the illuminating beam, it can be improved by using light of shorter wavelength than that of the visible spectrum, i.e. in the ultra-violet. The boundary between the two regions is not sharply defined, but we may take it as being at about 0·3 μ (see Fig. 1). The ultra-violet spectrum stretches away to shorter and shorter wavelengths, to another ill-defined boundary with the soft X-ray region at about 0·01 μ (100 Ångström units). This far u-v. is usually called the 'vacuum ultra-violet' region, as these wavelengths are so readily absorbed by the oxygen of the air that the microscope has to be evacuated. Even in the near u-v. the chief problem is to find lens materials transparent to the radiation, since ordinary glass hardly transmits any wavelengths shorter than about 0·3 μ. The material has to be easy to grind and polish, as well as being transparent, and those found most suitable are quartz, fluorite and lithium fluoride. With these materials it is possible to make an ultra-violet

microscope objective of short focal length and high N.A. Alternatively a concave mirror can be used as a reflecting objective; it has the advantage of being achromatic, and so it can be set up first in visible light. A special source of illumination is also needed. A cadmium or mercury vapour lamp, in a quartz bulb, is mostly used for the near u-v., having strong emission at wavelengths in the range 2,300–2,800 Å (0·23–0·28 μ).

With such equipment, in skilled hands, a resolving power of 800–1,000 Å can be attained; that is, twice as good as by the best visible microscopy. In this way we can explore finer details of cells and micro-organisms. Plate 3c shows an ultra-violet micrograph of locust sperm cells, for comparison with the results of ordinary optical microscopy (Plate 4). It fortunately happens that nucleo-proteins strongly absorb these u-v. wavelengths, enabling us to see details of the chromosomes in which they play an important part in determining heredity.

Attempts to use still shorter wavelength illumination run into increasing practical difficulties. Lens materials become less and less transparent, and suitable sources of radiation hard to make. Below about 2,000 Å it is preferable to abandon lenses and go over to a mirror system. A spark discharge between tin or zinc electrodes proves to be the best source of illumination. The whole microscope, including the specimen, has to be in an evacuated box or in an atmosphere of nitrogen. Microscopy can then be extended as far as a wavelength of 1,000 Å (0·1 μ). The specimens have to be very thin, owing to their heavy absorption, and images obtained at different wavelengths have quite different appearances, owing to the variation with wavelength of the absorption power of the various biochemical substances making up the specimen.

The still shorter wavelengths of the soft X-ray region present almost insuperable difficulties for microscopy, even by reflection from mirrors. Various alternative possibilities are beginning to be explored, as we shall discuss in Chapter 5. Curiously enough, microscopy turns out to be more feasible in the 'hard' X-ray region, at wavelength 1–10 Å, than in the soft region at wavelengths 10–1,000 Å. But even so, there are grave practical difficulties in obtaining a resolving power better than that of the ultra-violet microscope, as we shall see later.

The Ultra-microscope

It is possible to use a normal microscope of high power in such a way as to 'see' particles much smaller than its resolving power. If a strong beam of light is shone sideways into a liquid containing finely divided material in suspension, and the microscope is focused on a region below the surface (Fig. 26), very bright spots will be seen moving about the field of view. Similarly smoke particles can be seen in a box into which light

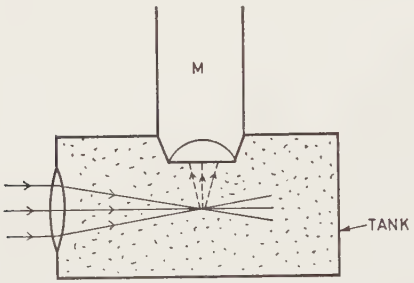

Fig. 26. The Ultra-microscope: light entering a smoke cloud, or a liquid containing fine particles, is scattered in all directions and a microscope at right angles to the axis may collect sufficient of it for particles to be seen which are much smaller than the resolving power as usually defined.

comes through a lens in one wall. Such an arrangement is known as an ultra-microscope, and was originally devised in 1903 by Zsigmondy. What is seen is the light scattered sideways by each particle, like a diffracted beam of high order. The degree of this so-called Tyndall scattering proves to be a maximum for particles about equal in size to the wavelength of the illumination, but particles much smaller than this are still visible. The microscope can detect them, but can give no information about their size. Resolving power has no meaning in this situation, since it is defined in terms of the separation of two particles on the axis of the illuminating beam.

Until the advent of the electron microscope, the ultra-microscope was the only means we had of detecting particles smaller than the resolving limit of the optical microscope. Particles as small as 4 mμ (40 Å) have been observed with the aid of illumination from a strong arc lamp. The method is really a special version of the general technique of dark-ground microscopy, in which the direct illuminating beam is prevented from entering the objective, usually by an opaque stop in the

centre of the lens. It is particularly valuable for observing the 'Brownian motion' of particles 0·1 μ to 1 μ in diameter, which is caused by the uneven forces exerted on them by the molecules moving about in a liquid or gas. In this way it gave the first direct, or almost direct, proof of the molecular motion which is the basic assumption in the kinetic theory of gases and liquids. By careful observation of the random paths followed by particles in Brownian motion, and with the help of a theory due to Einstein, it enabled the first reasonably accurate value to be obtained of Avogadro's Number, the number of molecules in a gram molecular weight of a substance.

Interference Microscopes

As pointed out earlier in this Chapter (p. 58), the ordinary type of microscope produces images in which details are visible because different parts of the object have different transparencies for the illumination used. The incident light intensity is reduced by a variable amount according to the absorbing properties of the substances present, and the image shows 'amplitude contrast'. So a specimen comprising constituents all of which are equally transparent will look featureless in the image, even though the refractive indices of the constituents may differ. However, there is an effect of the refractive index on a light beam which can be utilised to form a differentiated image in such circumstances: the light will be slowed up by differing amounts depending on the local value of the refractive index. In consequence, the emergent beams will differ in phase though not in amplitude. A system which converts the phase differences into amplitude contrast is termed an interference microscope, for the very good reason that it does so by means of interference effects. A special type of interference microscope, devised for certain biological uses, is the phase-contrast microscope.

Most of these new microscopes involve optical principles, such as polarisation and double-refraction, which are beyond our present scope, but the basic idea is simple. Consider two light waves, of identical wavelength λ_a in air, one of which goes through a material of refractive index μ whilst the other is

caused to by-pass it and continue through air on a parallel path (Fig. 27). The first wave will travel at lower velocity, since the refractive index of a material is equal to the ratio of

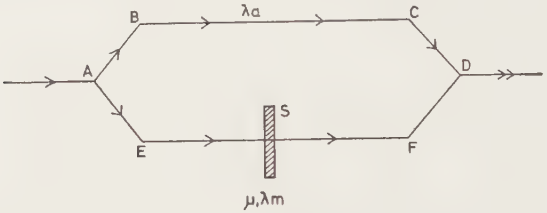

Fig. 27. Principle of the interference microscope: a beam of light is divided at *A* so that one beam travels the path *ABCD* in air and a second beam the path *AEFD* in which the specimen is placed. The two beams will not normally be in phase when re-united at *D*.

the velocity of light in air to its velocity in the material. The frequency of the light is unchanged, and so there will be more waves per unit length in the material than in air. If there are n waves in a path length d in air, there will be $n \times \mu$ waves in the same distance in the material. Alternatively, we see that the wavelength in the material (λ_m) will be shorter than in air, and in fact

$$\lambda_m = \lambda_a/\mu$$

This means that over a single wavelength this wave (wave 2) falls behind that in air (the 'reference wave', 1) by a definite amount, as shown in Fig. 28. A crest of one wave-motion is retarded with respect to that of the other, or, in terms of vibrations, the phase of the former lags behind that of the latter, by an amount depending on the refractive index.

It is interesting to calculate the amount of the phase shift in a typical specimen. Since the wavelength in a medium of refractive index μ is reduced to λ_a/μ from its value in air, λ_a, the lag $\triangle\lambda_a$ occurring in a path length of one wavelength is given by

$$\triangle\lambda_a = \lambda_a(1 - 1/\mu) = \lambda_a(\mu - 1)/\mu$$

or

$$\triangle\lambda_a/\lambda_a = \triangle\mu/\mu$$

Tissue cells have a refractive index very close to that of water,

1·33, and so the value of $\triangle\lambda_a/\lambda_a$ will be $0{\cdot}33/1{\cdot}33 = 0{\cdot}25$. In other words, a crest of the wave passing through the cell falls behind a corresponding crest of the wave in air by one-quarter wavelength in an air-path of one wavelength. If the air-wavelength of the illumination is 0·5 micron, then in a tissue

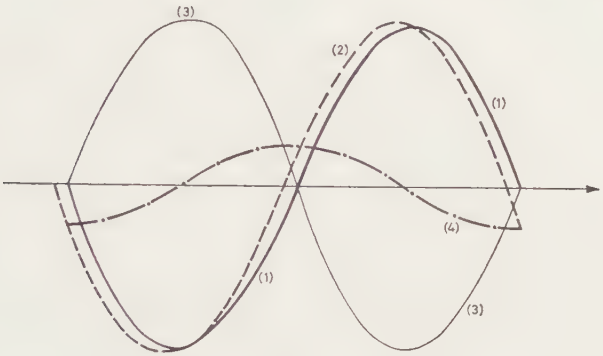

Fig. 28. Mutual relation of waves in the interference microscope: the wave (1) incident on the specimen is retarded slightly in passing through it, giving wave (2). When wave (2) is mixed with wave (3), which is obtained by shifting by a half-wavelength the twin wave to (1) which did not pass through the specimen, the resultant wave (4) has an amplitude which depends on the amount by which (2) was retarded. (*From* Progress in Microscopy *by M. Françon; Pergamon, Oxford.*)

section 2 microns thick the lag will become a complete wavelength.

More usually we are comparing different parts of a specimen with each other, and not with a reference beam in air, so that we should discuss the problem in terms of the variation of refractive index within a tissue cell, or between the cell and the medium in which it is mounted. If $\triangle\mu = 0{\cdot}1$, as it may well do, then the lag per wavelength (the 'phase-shift') will be only $0{\cdot}1/1{\cdot}33 = 0.075$, less than one-tenth of a wavelength. If $\triangle\mu = 0{\cdot}04$, the shift is only 0·03, so that even in a 2 micron section the shift is only about one-eighth of a wavelength. This value is typical of much biological material, and the fact that it is so small is basic to the design of the phase-contrast microscope, described on p. 80.

If we had a phase-sensitive detector, we could measure directly the phase-shift caused by a specimen detail, but this is not practicable with light. We have to find means of transforming the phase-shift into an amplitude change. In principle this can be done very simply: by changing the phase of the reference wave by exactly a half-wavelength. By inverting the phase of wave 1 we obtain wave 3 (Fig. 28). This new wave would be completely cancelled by wave 2 if the latter had not been retarded, but in fact this retardation leaves a resultant wave of small amplitude (4) when we now add waves 2 and 3. That is, we end up with a detectable light signal, the strength of which will depend on the difference in refractive index between the path in the specimen and the reference path in air. The phase lag will also depend on the total path length: it will increase with each wavelength travelled, and will only be equal to that shown in Fig. 28 if the specimen is a whole number of air-wavelengths thick. If the thickness were such that wave 2 came through shifted by exactly a whole number of wavelengths, it would cancel completely wave 1, if this had been shifted by a half wavelength as we supposed. So clearly we need a device which allows us to shift the reference wave by any amount we choose, until we get maximum contrast between the two waves which interfere in the final image.

An interference microscope, therefore, is an optical system which splits the illumination into two beams and provides a means of shifting the phase of one of them by a variable amount. The many different types of interference microscope differ mainly in the means by which these two requirements are met. The phase shift is most easily controlled by pushing a wedge of glass across the reference beam, thus changing the path length with respect to that in the specimen. The simplest beam-splitting arrangement is that in which two entirely separate paths are followed, with an objective lens in each and the specimen in one of them (Fig. 29); a phase-shifting device is placed in the reference beam. This is known as the Mach-Zehnder principle and is used in the Leitz interference microscope, which is thus a double-barrelled instrument. Two half-silvered mirrors are used to split the beams on entering the

system and to re-combine them before they enter the eye-piece. The Dyson microscope is a method of using a concave mirror, a plane mirror and two half-silvered mirrors to produce the

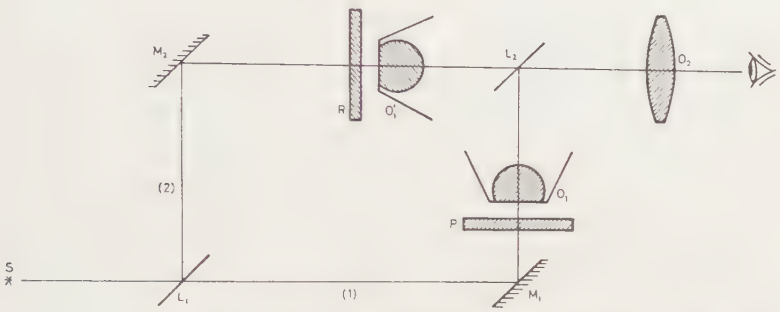

Fig. 29. A double objective type of interference microscope. Light from the source S is split by the half-silvered mirror L_1 into two beams which travel separate paths through the matched objectives O_1 and O'_1 before being re-united by the half-silvered mirror L_2. The specimen P is placed in one beam and a phase-shifting plate R in the reference beam. (*From* Progress in Microscopy *by M. Françon; Pergamon, Oxford.*)

same result in a single objective (Fig. 30). The more complicated Smith microscope utilises doubly-refracting prisms, a polariser and an analyser, and there are many other designs.

Complicated as the interference microscope may be in its construction, it is not much more difficult to use than the normal type of microscope. As already mentioned, its great value is the ability to discriminate between the various component parts of living matter. These are often equally transparent to light, and so quite invisible by normal microscopy. In the interference microscope differences in refractive index show up at once, and the contrast of each component in turn can be maximised by adjusting the phase-shifting device. Specimens which are featureless in an ordinary microscope may reveal a wealth of detail in an interference microscope, as the example in Plate 6A, B shows. The importance of the new instrument for the study of living cells can hardly be exaggerated, since it allows us to follow their development at high magnification over long periods of time. In this way some striking films have been made of the details of cell division, for

instance, and, in quite a different direction, of the growth of crystals from a solution. It can also be adapted for viewing surfaces, as required for metallography.

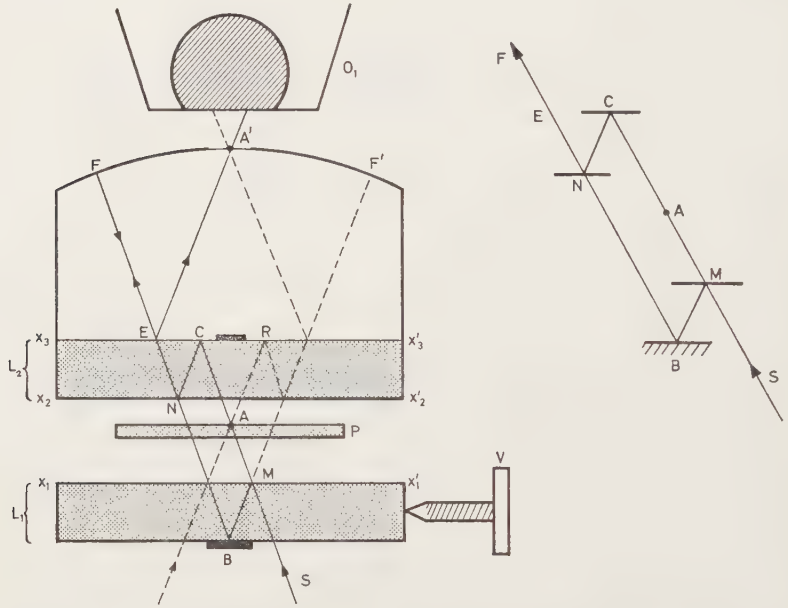

Fig. 30. A single objective type of interference microscope; simplified ray paths (right) and complete system (above). The light beam S from the source travels two different paths defined by the half-silvered surfaces (M, N, E), the fully-silvered discs (B, C) and the concave mirror (F), before passing through the aperture at A' into the microscope objective O_1. P is the specimen and L_1 is the slightly wedge-shaped phase-shifting plate. (*From* Progress in Microscopy *by M. Françon; Pergamon, Oxford.*)

The Phase-contrast Microscope

The interference microscope, as we now know it, is a post-war development and its use is still not very widespread. It was preceded by the phase-contrast microscope, the principle of which represented the greatest advance in microscopy since the 19th century, when it was discovered by the Dutch physicist Zernike in 1933. It is a much simpler instrument than the interference microscope both in design and in operation, and it is now widely used in biological laboratories.

Basically its design depends on the circumstance, mentioned above, that the differences in refractive index between the different parts of a cell are so small that the total phase shift, even in a relatively thick section, is still only a fraction of a wavelength. In consequence, the emergent beams will be approximately in the relation of waves (1) and (2) in Fig. 28, the starting point of our discussion of the interference microscope. This shift can also be regarded as due to the production of a new wave (4), which combines with the original wave (1) to produce wave (2). In constructional terms, we 'resolve' (2) into two components, one of which is the original wave and the other a wave of small amplitude (4), shifted by a quarter wavelength with respect to (1). As we might suspect from our earlier discussion, it is found that maximum visible (or 'amplitude') contrast occurs if wave (4) is shifted by a further quarter wavelength, so as to be either exactly in phase or out of phase with the original (reference) wave. So a fixed phase shift of this amount is built into the phase contrast microscope. Instead of splitting the beam with some special device, advantage is taken of the fact that two separate beams are produced by diffraction in the specimen, in the shape of the zero-order beam and the first-order diffracted beam (see Fig. 24). So all that is needed is to put a phase-shifting plate in one of these beams, either as a small disc at the axis in the direct beam or as a ring in the outer zone where the diffracted beam passes (Fig. 31). The phase plate, which is of etched glass, may be made to retard either the diffracted or the direct beam, giving positive or negative phase contrast, respectively. Whether a particular specimen detail appears bright on a dark background, or vice versa, will depend on whether its index of refraction is greater or less than that of its surroundings, as well as on the type of phase contrast.

The appearance of an image in these two types of phase microscopy is shown in Plate 6c, d. Also visible is the halo which surrounds thick parts of the object even when in best focus. It arises because the separation of the two beams is not perfect, and constitutes the main disadvantage of the phase-contrast system. The main advantage, with respect to the split-beam interference microscope, lies in the simplicity of its construction

and use. Once the phase plate is centred and the illumination properly aligned, there is no further adjustment to be made. It has been found useful in a great variety of applications,

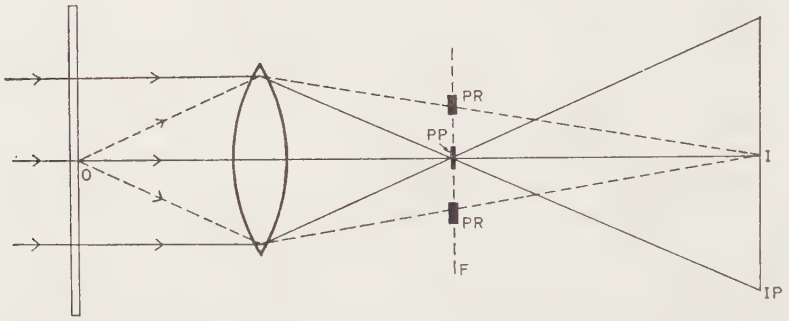

Fig. 31. Principle of the phase-contrast microscope: some of the light falling on a small object *O* is diffracted (broken lines) and brought to a focus in the corresponding image point *I*. The undiffracted light (full lines) is focused in the back focal plane of the lens, and is then spread over the image plane *IP* as a uniform background. A phase stop may be placed there (at *PP*) or a phase ring (*PR*) at a radius which will intercept the main part of the diffracted beam.

especially in biology, despite the lack of the versatility provided by the variable phase shift in the interference microscope proper.

Postscript on the Use of the Compound Microscope

The most important advice, in starting out to use a microscope for the first time, is to follow the instructions in the manufacturer's handbook. If you have come by a second-hand, rather than a new, instrument it is worth writing to ask the makers if an instruction book is available for it. Unless the microscope is very long out of date, they may well be able to supply a copy. But even so, instructions are not always complete, and if they are, they may not set out the reasons for the various procedures to be followed. So it will be worth while to read over the relevant chapters in one of the many

practical manuals of optical microscopy, to make sure that you are getting the most out of your instrument. One of the best short accounts will be found in the *Use of the Microscope*, by R. Barer (Blackwell's Scientific Publications, Oxford).

The bare bones of the proper procedure for setting up a microscope are as follows:

1. Raise the tube which carries the lenses until its lower end is well above the specimen stage. Insert a low-power eye-piece and low-power objective lens. The condenser should be in position, with its iris diaphragm wide open.
2. Place the light source some way from the mirror at the foot of the microscope; 10–12 inches is a good distance. It should be a lamp with a frosted, not a clear, bulb. Look through the eye-piece and swivel the mirror until a blaze of light is seen.
3. Place a specimen on the stage and lower the tube by means of the coarse focusing knob until the front of the objective is close to the specimen. Then, looking through the eye-piece, turn the same knob slowly so as to raise the tube and bring some feature of the specimen into rough focus. Use the fine control to bring it into exact focus, and then adjust the mirror, if necessary, so that the field of view is uniformly illuminated.
4. Now raise or lower the condenser lens, by means of its control knob, until the frosted surface of the bulb appears in the field of view. Raise the condenser just a little more, so that the pattern vanishes. To get the optimum conditions for resolving power, remove the eye-piece drawtube, look into the main tube of the microscope, and close the iris diaphragm of the condenser until its edges are just visible around the rim of the objective. Replace the eye-piece.
5. Examine different parts of the specimen by operating the stage controls, to find features of special interest, and then replace the low power objective with one of higher power. If your microscope has a rotating nosepiece carrying several objectives, this is simply

done—but do it cautiously on the first occasion, in case the rim of the objective does not clear the specimen, as it should do. Only a slight adjustment of focus should be necessary. If your microscope takes only a single objective, raise it well above the specimen, unscrew the low power lens and insert one of higher power. Repeat the focusing procedure.

6. The condenser must now be readjusted: on looking through the microscope you will see that the image is much less bright than it was and details are vaguer. On removing the eye-piece, only the centre of the objective will be seen illuminated, so open up the iris until its edges reach the rim of the objective again. Replace the eye-piece—and you will be surprised at how bright and sharp the image has become.
7. Repeat steps 5 and 6 with a still higher power, if the specimen has very fine details which warrant examination at very high magnification.
8. As with any other pursuit, practice makes perfect and brings greater pleasure and better results. Half the key to success with the microscope is knowing what to look for! The first time you use it you may find yourself looking at scratches on the slide which carries the specimen, or dirt on its surface, and not at its true structure at all. It is as well to start with some very simple specimens, the details of which are known, such as are frequently supplied with a new microscope. If you have an old instrument, a hair or a piece of printed cellophane will serve to give you practice in the focusing procedure. Once you are familiar with the routine, it should soon become automatic and you will be sure of getting the best out of your microscope.

CHAPTER

4

THE ELECTRON MICROSCOPE

In an electron microscope the specimen is illuminated with an electron beam, instead of a light beam, focused by electron lenses consisting of magnets or electrically-charged plates so as to form a highly-magnified electron image. Just as in a television tube the electron beam has to be produced and focused in a vacuum, the image appearing on a specially-coated screen, which now, however, does not form the end wall of the instrument. When the image has been focused, the screen can be lifted and a plate exposed in a camera below it, providing a permanent photographic record. In general principles the electron microscope is thus very similar to the optical microscope, comprising a source of illumination, a condenser system and two (but sometimes three) stages of magnification (see Fig. 6). The main difference is that the final lens has to project a *real* image on to a viewing screen, and so is called a 'projector', since the eye cannot receive the electron image direct. In practice, however, the construction and appearance of the instrument is quite different (Plate 8). It stands some 6 feet high, instead of about 18 inches, it requires vacuum pumps, a high-voltage supply of 500,000–100,000 volts and other complicated electrical equipment, and it costs £10,000 or more instead of £100 or less.

In return for this cost and complication we would expect a correspondingly higher performance, and we are not disappointed. Where the best light microscope has a limiting resolving power of about 200 mμ (0·2 μ) and the ultra-violet microscope one of 100 mμ, the standard electron microscope now goes down to 1 mμ (10 Å) and specially 'tuned-up' models almost to 2 Å. Indeed, the improvement in resolution

is roughly proportional to the increase in price, although it must be said that there are cheaper electron microscopes and more expensive optical microscopes than the figures quoted.

It is remarkable that this great advance in the power of our tools for studying the microscopical world has taken place in no more than thirty years. It was made possible by a steady accumulation of discoveries and technical skills, followed by the sudden realisation that it would be possible and fruitful to construct a microscope for electrons. Technically it would have been feasible to make such an instrument some thirty years earlier still, but the key idea was lacking. Electron beams had been produced since the late 1850's and electrical engineers had known since 1899 how to focus the beam in their early cathode-ray tubes by means of coils wound around the neck of a tube. However, electrons were looked on as negatively charged *particles* at that time; it was only when they were shown to be *waves* of very short wavelength that their value for microscopy became clear. The essential idea of the wave nature of the electron was put forward as a hypothesis by L. de Broglie (in Paris) in 1924 and its correctness was proved when G. P. Thomson (in Cambridge) in 1927 obtained diffraction patterns when an electron beam passed through a thin metal foil. It was soon shown that the electron wavelength was very much shorter than that of visible light, and even smaller than that of X-rays. Within a few years Knoll and Ruska (in Berlin) had built the first electron microscope and in 1933 they produced photographs showing a resolution better than the limit of the optical microscope. The first commercial model was put on the market just before the Second World War, and by the present time there are something like 3,000 electron microscopes in use throughout the world, the most active country being Japan. As in most branches of modern science, the development of the electron microscope has been international in its scope, and characterised by a curious mixture of keen rivalry and friendly exchange of ideas.

A proper description of the electron microscope must start with some account of how beams of electrons can be produced and controlled, and go on to the basic principles of electron

lenses. Fortunately the radio valve and television tube have now become common features of everyday life, so that most of the ideas will not be entirely strange to the reader. We then discuss the special requirements of electron lenses for use in a microscope, followed by the main factors in the design and construction of the instrument, and some account of the present limitations on its performance. Some examples will be given of the new insight it is giving us into the ultra-fine structure of Nature, in the animal, vegetable and mineral kingdoms.

Electron Guns

The easiest way of getting an electron beam is to heat a piece of metal wire strongly, whilst applying a positive voltage to a metal plate placed near to it. Electrons are given off from the wire and, being negatively charged, are attracted to the positive plate. This is the arrangement in the simplest type of thermionic valve—'thermionic', because the electrons are produced by heating, 'valve', because they pass in one direction only, from the negative to the positive 'electrode'. These are termed the cathode and anode, respectively, and the tube as a whole is a diode. It has to be evacuated of all air, down to a very low pressure, since electrons are very readily stopped, even by a gas. The output of electrons increases very rapidly with temperature, and so it is best to use as filament a metal which can be heated to a high temperature without melting. Tungsten is usually used in electron microscopes; it has a melting point of about 3,300°C. For radio valves and cathode-ray tubes, where a long life is important as it is not easy to replace the filament, a nickel bar or plate coated with a special mixture of barium and calcium oxides is employed, which also has the advantage of operating at much lower temperature. By applying a high enough voltage close enough to a metal filament, it is indeed possible to get a small amount of electron emission even when it is at room temperature, as we shall see in the next chapter, but it is not a practicable system for electron microscopy.

By boring a hole in the anode we can allow electrons to pass through as a beam. A diode is not very efficient for this

purpose, however, since electrons from the filament are attracted to all parts of the anode and only a fraction of them pass through the hole. The solution is to add a third electrode, (*B*), negatively charged (or 'biased') with respect to the cathode, (*C*), between *C* and the anode (*A*) as shown in Fig. 32. The negative voltage on *B* repels the stream of electrons towards the axis, an action which is assisted by making the

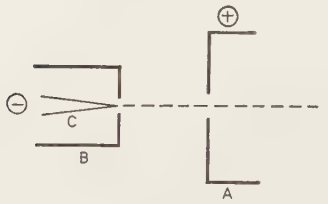

Fig. 32. Triode electron gun: *A* = anode, *B* = bias electrode, *C* = cathode.

filament in the form of a hairpin, so that electrons are emitted only from a point on the axis. This system is exactly similar to a slope down which ball-bearings are rolled towards a hole in a wall at the foot. A suitably shaped gully or trough, running towards the hole, will help to funnel the balls into it: a ball which deviates from the line of fire will rise up on the side of the gulley for some way, before turning back towards the axis. Such an arrangement forms a 'gravitational lens', just as the triode system of cathode, bias electrode and anode forms an electron lens. The bias electrode, *B*, corresponds to the 'grid' of a radio valve. In the electron microscope it is usually made in the form of a cylinder, closed at one end except for a small aperture in which the point of the filament is situated; it is sometimes called the 'Wehnelt cylinder' after the man who discovered its action.

It is rather a delicate matter finding the correct voltage to apply to *B*. If it is too high (negatively) it will cut off the emitted electrons altogether, setting up a barrier which they do not have enough energy to surmount. If it is too low, the electrons will still spread too widely over the anode. The designer can get a rough idea of the right value by making a scale model of a cross-section of the electrodes, with heights proportional to their negative voltage (taking the anode as zero), and stretching a rubber sheet over them, pinning its

edges down to a wooden frame. By releasing ball-bearings from the 'cathode' and adjusting the height of the 'shield', he can find the right set of conditions for guiding them into the 'anode' aperture. This gives him an idea of the voltage to apply to the actual shield when the anode voltage has a particular value. In practice, he must provide for the shield voltage to be varied over a wide range, both because the rubber model is not an exact one and because the microscope user will want to operate at different anode voltages for different types of specimen.

Such a triode system is called an 'electron gun', as it is designed to direct a beam of electrons at high speed along a predetermined path. The tungsten filament is heated either by a battery, or by a transformer supply, to white heat. The anode voltage is usually variable over a range of two or three times, from 50,000 volts (50 kV) to 100 kV or from 30 kV to 80kV, and is obtained from a transformer-rectifier supply. For reasons which will appear later, this high voltage has to be stabilised to one part or so in 100,000, which requires complicated electronics and adds greatly to the cost of the microscope. The bias voltage applied to the shield will depend on the size and separation of the electrodes, but will mostly be in the range 200 to 2,000 volts. The beam current from such an electron gun is surprisingly small, of the order of 10 microamperes (0·00001 amp.), but this is amply sufficient for most conditions of microscopy, except at very high magnification.

The electrical supplies are usually generated in a separate cabinet and fed to the microscope column by a high-voltage cable, as seen in Plate 8. The various controls and meters are laid out on the microscope desk, so that the operator can select the desired anode voltage and adjust the filament and bias voltages at will. Interlocked safety devices are normally provided in the circuits, to prevent the high voltage being applied until the column of the microscope has been brought to the necessary high vacuum by the pumps. If the anode voltage were to be applied whilst air was still in the column, it would flash over to the shield and overload the supply circuits.

Electron Lenses

The electron beam issuing from the anode serves as illumination for the electron microscope proper. It is focused on to the specimen by one or more condenser lenses, and a highly-magnified image is then produced by an objective and one or more projector lenses. To keep the overall length of the instrument to a reasonable size, and at the same time to provide a top magnification of 100,000 times or more, these lenses have to be of short focal length. They also have to be variable in strength, since it is inconvenient to move either the specimen or the lens along the axis for focusing the image. Fortunately the electrical or magnetic fields, which are our only means of focusing electrons, are readily varied by changing the voltage applied to the electrodes or the current in the magnet coils respectively.

Electrostatic Lenses are the simplest to make and to understand. The three-electrode system of an electron gun, just described, is in fact an example. The essential features are two or more electrodes, in the form of tubes or apertured discs, to which variable voltages can be applied. In the gun of a television tube it is usual to have several anodes at successively higher voltages. For electron microscopy a symmetrical three-electrode system is used, with the two outer ones earthed and the central electrode connected back to the cathode of the electron gun. In terms of the rubber model, the electrons have to travel up a slope again, almost to their starting height, and over a saddle before running downhill once more. The trough at the top is deep and narrow and, because the electrons are travelling very slowly through it, the focusing action is very strong, so that lenses of very short focal length can be made.

This type of lens was used in a number of electron microscopes in the early days, but as the demand grew for higher voltages and greater magnification, requiring stronger lenses, it was found that the electrostatic lens was inadequate. The chief reason is the danger of flash-over as the voltage on the electrodes is raised, even when the vacuum is good. This trouble can be avoided by allowing a bigger separation between

the electrodes, but then the focal length increases again. Even with lenses of moderate strength there is always the danger of a very small vacuum leak admitting enough air to cause flash-over. For these reasons the few firms which formerly manufactured electrostatic electron microscopes have now gone over to the use of magnetic lenses.

The *magnetic electron lens* functions on quite different principles, and a gravitational model is no longer helpful. Its action can be understood, however, on the basis of one of the elementary rules of electromagnetism, which will be familiar to most schoolboy readers, if not to their elders: the 'left-hand' or electric motor rule. It describes the action of a magnetic field on a moving electron: when the direction of the field is at right angles to the direction of motion of the electron, the force on the electron is at right angles to both. In other words, if we have a uniform magnetic field in the x-direction (Fig. 33) and an electron starts off in the y-direction, it will

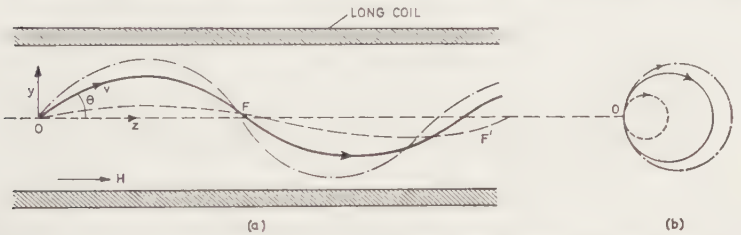

Fig. 33. Focusing action of the magnetic field inside a solenoid coil. Electrons emitted from O in the directions shown will be deflected out of the plane of the diagram by the uniform magnetic field H and follow spiral paths, which would appear as circles if seen from the end of the coil, as at (*b*). (*From* Introduction to Electron Optics *by V. E. Cosslett; University Press, Oxford.*)

experience a force in the z direction, i.e. into the plane of the diagram. It will still be cutting the field at right angles, however, and so the force will continue to bend it round in the y-z plane. In fact its resulting path will be a circle in this plane, the radius R of which will depend on the strength of the magnetic field H and the velocity v (or the voltage V) with which the electron was emitted from the electron gun: the stronger the field the smaller is the radius, whilst the voltage has the opposite effect.

If, now, the electrons are injected almost in the direction of the x axis, making a slight angle (θ) with it, instead of at right angles to it, their motion will be rather more complicated. We can regard their starting velocity as made up of two components, along the x- and y- directions respectively. The former, v_x, is not affected by the magnetic field since it is in the same direction. The latter, v_y, however, is at right angles to the field, so that there will be a force in the z-direction turning the electrons in a circle, as before. Since this circular motion is going on at the same time as the uniform motion in the x-direction, the net result is that the electrons follow spiral paths as shown in Fig. 33, returning to the axis after one complete turn. Viewed along the axis, the paths would all appear as complete circles, as seen at the right of Fig. 33. All electrons starting from O with the same velocity and the same direction will come back to the axis at the same point, X, but the distance OX will vary with the velocity and the angle θ. In other words the magnetic field forms a series of images of an emitting point, spread out along the axis. Anything like a single point image can only be obtained if we ensure that the electrons are all emitted with the same velocity (or voltage) and with very little variation in angle. Although the 'long field', as it is called, has a focusing action in this restricted sense, it is not a true lens because it has no proper focal length. Electrons which enter parallel to its axis are not deviated at all: by the left-hand rule there is no magnetic force on them. So, on the usual definition, the focal length is infinitely great.

A more effective lens is formed by a 'short field', which is produced by a slab coil and so is anything but uniform (Fig. 34). The focusing action is basically the same as in the long field: at any given point the component of the electron velocity which is at right angles to the local direction of the magnetic field gives rise to a force at right angles to both. An electron will therefore spiral round the axis as before, but with a radius of curvature which changes as it goes through the field. In general it will arrive back at the axis without having made a complete turn, as shown at the right of Fig. 34. Provided that all electrons have the same voltage and that the angle θ is small

(less than 1° in practice), a point image I will be formed of a point object, O, and more important, electrons entering parallel to the axis will also be focused, because they are not now travelling in the same direction as the field.

Such a magnetic lens, in fact, has very much the same

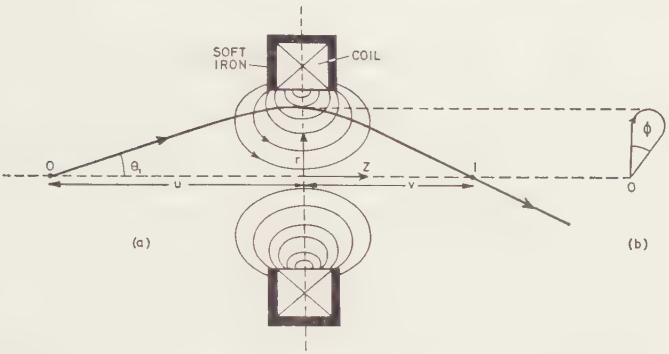

Fig. 34. Focusing action of a short current-carrying coil, the magnetic electron lens. Electrons from O are twisted about the axis by the magnetic field within the coil, crossing the axis again at the image point I. Seen from the end of the axis the paths are curved in the field, but straight outside it, as in (*b*). The lens action is strengthened by surrounding the coil with a casing of soft iron, as shown. (*From* Introduction to Electron Optics *by V. E. Cosslett; University Press, Oxford.*)

imaging properties for electrons as a glass lens has for light. So long as both the object and the image distances are much bigger than the extent of the field, it obeys the 'thin lens' relation between focal length f, object distance u, and image distance v:

$$1/f = 1/u + 1/v$$

When we need a high magnification, u has to be small and O will be inside the field, and the thick lens relations of optics are then obeyed. The chief difference between a magnetic lens and a glass lens is that the effective refractive index now changes from point to point through the lens, instead of being a constant for the lens as a whole. So there is no such simple relation between focal length, refractive index, and radius of curvature as we had for an optical lens (p. 36). The best we

can do is to relate f to the strength of the magnetic field at the centre of the lens, H_{max}, to the internal radius, R, of the coil or electromagnet producing it, and to the voltage, V of the electrons as they enter it. By careful measurements it is found that the focal length is given by

$$1/f = aH^2_{max}R^2/V$$

where a is a factor which is approximately constant except in very strong lenses. The value of H_{max} in a lens of given radius R is determined by the 'excitation' NI, the product of the number of turns, N, in the lens coil and the current, I, flowing in it. So a more useful form of this relation is

$$1/f = \beta\ (NI)^2/V$$

where β is another factor, which depends on the design of the lens.

A strong lens, of short focal length, thus needs a strong magnet field and small lens radius R. It is found that for a

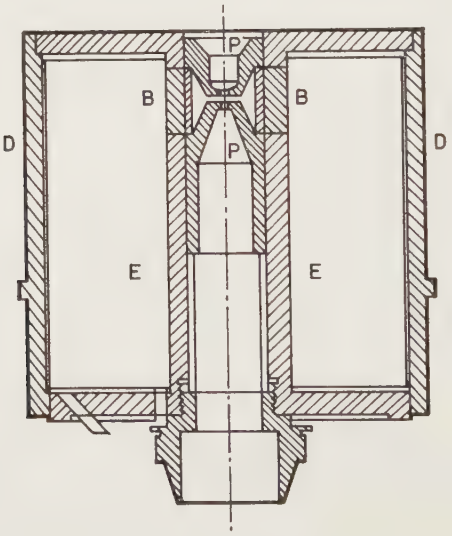

Fig. 35. Cross-section of magnetic lens as used in the electron microscope. E = coil winding, D = soft iron casing, B = non-magnetic spacer, PP = pole-pieces of soft iron.

given excitation, NI, the value of H_{max} can be forced up by compressing the field into the centre of the lens. This is done by surrounding the coil with soft iron and fitting pole-pieces inside it, separated by a small gap, S (Fig. 35); at the same time the pole-piece radius is made as small as is practicable, a few mm. The value of the factors α and β are then found to depend on the ratio of gap to radius, S/R, but the variation is small in strong lenses. As an example, with a field strength of 5,000 oersted and a gap and radius each of 3 mm., a 60 kV electron beam would be focused with a focal length of 4 mm. Such a field could be produced by an excitation of approximately 2,000 ampere-turns; i.e. a current of 1 amp. in 2,000 turns or of 0·1 amp. in 20,000 turns and so on. Stronger lenses have been made, but the problem arises of getting rid of the heat generated by the current in the lens coil. The objective in most electron microscopes has to be cooled by means of a water jacket.

The reason for operating at as short a focal length as possible is partly to keep the microscope compact, and partly to keep the spherical and chromatic aberrations down to tolerable proportions, as we shall see later. The strongest objectives so far made have a focal length of slightly less than 1 mm., rather shorter than the most powerful glass lens available for the optical microscope. This is the *minimum* focal length, of course, since the strength of a magnetic lens can be varied over a wide range by changing the current in its windings. A specimen in an electron microscope can thus be brought into focus simply by turning the control knob of a resistance in the lens circuit; it is no longer necessary to move the lens itself, as in the light microscope.

Design of the Electron Microscope

The complete lay-out of the column of a modern electron microscope is shown in Fig. 36. The electron gun, G, is followed by two condenser lenses, C_1, C_2, which direct the beam on to the specimen at S. The first condenser is a strong lens, usually operated at full strength, which forms a demagnified image of the cathode inside its lower pole-piece. This small

electron spot is imaged at a magnification of about 2 in the vicinity of the specimen by C_2, a weak magnetic lens of long focal length. By varying the strength of C_2, we can control the size of the illuminating spot on the specimen, and by changing the size of the aperture in C_2 we change the total beam current

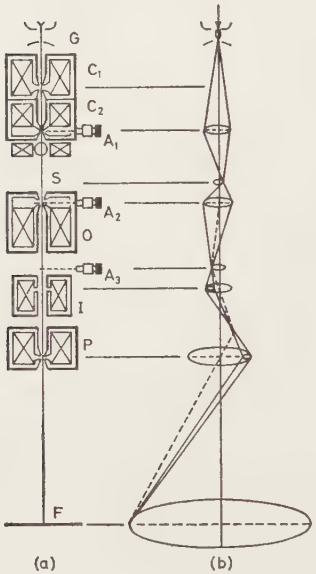

Fig. 36. A modern electron microscope (*a*) in schematic cross-section and (*b*) showing how the electron image is formed. G = electron gun, C_1 and C_2 = first and second condenser lenses. O = objective lens, I and P = intermediate and projector lenses, S = specimen, F = fluorescent screen, A_1, A_2, A_3 = beam limiting apertures.

falling on S. It is important to be able to do this, because the beam heats up a specimen and some are more susceptible to damage than others. Also there is no point in illuminating S over a larger area than will be imaged on the viewing screen; electrons outside this area do not contribute to the image, but simply heat up the specimen.

The imaging system consists of the objective lens, O, the intermediate projector, I, and the final projector, P. Most of the magnification takes places in O and P, and I is mainly employed for varying the overall magnification. The objective is a very strong lens, specially designed to have minimum aberration and usually fitted with a device called a 'stigmator' for correcting astigmatism. A very small aperture can be inserted into its back focal plane for the purpose of limiting the spherical aberration, as described later. An image of S is

formed by O just above the pole-piece of the intermediate lens, I, which in turn projects a further enlarged image into the second projector, P. Then P projects the final image on to the fluorescent viewing screen, F, which the operator can see through a window near the foot of the column. Beneath F is a camera box containing a number of plates for photographing the electron image direct when F is lifted; alternatively roll film can be used, but plates are generally considered to give better results.

The microscope column must be kept compact, so that the operator can make necessary adjustments to various parts. The distance from cathode to screen is normally about 1 metre, with the specimen about half way between G and F. As the column sits on the control desk, the cathode is 6 feet or more from the ground. The high voltage is fed to it by a cable with its outer casing earthed, so that there is no danger to the operator. In the very high voltage microscopes which have recently been built, going up to 500 kV and even 1 MV, all the dimensions are necessarily bigger and the operator has to use remote controls.

The top magnification in high performance microscopes may be as much as 200,000 ×, divided between the lenses O, I and P as 100 ×, 20 × and 100 ×, respectively. At full strength the specimen must be close to the front focal plane, so that the object distance, u, is about equal to the focal length and the magnification is about v/f. So if the minimum focal length of the objective is 2 mm., the image distance v will be about 20 cm. for $M = 100$, and the situation will be much the same in the final projector, P. If these were the only two imaging lenses, the top magnification would be only $100 \times 100 = 10{,}000$ times, unless each stage was made longer. To get a top magnification of 200,000 × in a two-stage microscope would require a specimen-screen distance of about 2 metres. The need for the intermediate lens is thus clear. If its focal length is no smaller than 1 cm. it can still provide the extra 20 times magnification in an extra length of only about 20 cm. All modern electron microscopes of high resolving power are therefore made as in Fig. 36, with a double projector as well as a double condenser system.

The optical microscope is quite satisfactory with two stages of magnification, an objective and an eye-piece, because its maximum useful magnification is no more than 3,000 ×, even with ultra-violet light. In the electron microscope, the maximum useful magnification is over 100 times greater. As we mentioned on p. 65, the value of M_{max} is given by the ratio of the resolving power of the eye to that of the microscope. Taking the former as 0·2 mm. and the latter as 5 Å (0·5 mμ), we get $M_{max} = 400{,}000$ for a fully-tuned-up electron microscope. Such a very high electron magnification could be provided by making the column long enough or by adding a fourth stage of magnification, but the image on the viewing screen already begins to get very dim at 100,000 ×. Since the image on the plate can readily be enlarged another 5 or 10 times, the solution adopted is to provide the operator with a viewing telescope of 5 to 10 × magnification for observing the image on the screen. It is preferable to use it even when working at less than top magnification, so that the electron beam intensity on the specimen can be reduced. The overall arrangement is thus one of four-stage magnification, three electron-optical and one light-optical.

Practical Limitations on the Resolving Power

The figure of 5 Å just quoted for the resolving power of an electron microscope is very much worse than would be expected from the size of the electron wavelength, λ_e. As measured in diffraction experiments, λ_e is related to the beam voltage by the approximate expression:

$$\lambda_e = \sqrt{(150/V)}$$

giving λ_e in Ångström units when V is in volts. So an electron beam of 60 kV will have a wavelength of about 0·05 Å, and, as we saw in connection with the optical microscope (p. 71), the absolute limit of resolving power should be about one-third of the wavelength, i.e. 0·02 Å or so. However, that conclusion rested on the assumption that diffraction was the only defect

limiting the sharpness of the image, and that all lens aberrations could be corrected sufficiently well for the full aperture to be used. The perfection of lens design which makes this possible is based upon 200 years or more of experience of aberrations and their correction. Electron lenses have been under active investigation for less than forty years, and we are as yet very far from being able to reduce appreciably the severe aberrations from which they suffer. In consequence, the main factor which limits the resolving power of electron microscopes is not diffraction but spherical aberration.

The problem of correction is made difficult by the fact that all ordinary electron lenses, whether electrostatic or magnetic, have positive spherical aberration. That is, the outer zones of a lens have a stronger focusing action than the central zone, as in Fig. 18. There are no simple lenses with negative aberration which could be combined with one of positive aberration to give a corrected system, as is possible in glass optics. In principle there are one or two complicated methods by which correction might be practicable, but so far they have not been made to work well enough for use in an actual electron microscope.

The effect of spherical aberration increases rapidly with lens aperture, α. The diameter of the disc of confusion in the image of a point object is proportional to α^3 in fact. So we are able to keep the image sharp by using a very small aperture in the objective. If we make it too small, however, we run into the effect of diffraction, which we saw earlier (p. 70) is inversely proportional to α. As we stop down the aperture to reduce the spherical aberration, the size of the blur due to diffraction begins to increase. The compromise usually adopted is to select an aperture for which the two effects are just equal. At the usual working voltages of 50–100 kV, the optimum aperture turns out to be about $\frac{1}{2}^\circ$ as compared with a value of over 80° in the best optical lenses. For a focal length of 2 mm., this corresponds to a hole of diameter 0·04 mm. (40 μ) at the back focal plane of the objective lens.

The smallness of this aperture requires the utmost care in setting up an electron microscope. The aperture must be exactly on the axis of the objective, which in turn must be in

line with the other lenses. The aperture in the condenser system will be not much larger than that in the objective, whilst those in the intermediate and projector lenses can be considerably bigger. The main parts of the microscope column are sufficiently well aligned with each other by the manufacturer, leaving the operator to line up the condenser system and the objective. He is able to centre the aperture in the latter by means of micrometer drives at right angles to each other. The condenser may then be shifted bodily, or the beam may be deflected electromagnetically as in a cathode-ray tube, until it is properly directed into the objective aperture.

An advantage of the small aperture is that it reduces the other lens aberrations as well as the spherical one, and in particular the chromatic aberration which arises from the fact that the focal length of a magnetic electron lens depends on the beam voltage and on the current in the windings. It cannot be kept negligible by use of a small aperture alone, and so we have to provide elaborate electronic equipment to keep stable the high voltage and the lens current supplies. This has to be done to within one part in 100,000 in a high performance microscope.

Similarly the astigmatism may not be made small enough by the use of an aperture, but this aberration can fortunately be corrected directly by the operator. It is equivalent to the lens having different focal lengths in different longitudinal cross-sections, due to the magnetic field not being of uniform strength around the axis. The solution is to introduce compensating fields which are unsymmetrical in the opposite way, and a 'stigmator' is a device for doing so. With it, the operator is able to add an electrostatic or magnetic field in the right direction across the lens axis, in the back pole-piece, and to adjust its strength until the astigmatic effect in the image is removed. The stigmator also helps to correct for misalignment of the objective aperture and some other disturbing effects. Indeed, the resolving power of modern microscopes could not have been pushed to anything like its present value without the invention of the stigmator.

But as soon as one hurdle is surmounted, the operator finds others in the way of achieving the calculated resolving power. From the known values of the spherical and chromatic aberra-

tions, a resolving power of between 2 Å and 3 Å ought to be attainable at the usual working voltage of 50–100 kV. To obtain an electron image actually showing this resolution, all sorts of engineering problems have to be satisfactorily solved. In particular, the electron image must not move with respect to the object by more than this minute distance of 2–3 Å during the few seconds exposure needed to take the photograph. This clearly means paying close attention to the mechanical stability of the microscope column, but also to the thermal, electrical and magnetic stability: to the thermal stability, because the specimen is under strong electron bombardment, which heats it and may cause it to drift, owing to expansion; to the electrical stability, because scattered electrons will slowly charge up any insulated regions they may strike inside the column, giving rise to a slow drift of the beam; to the magnetic stability, because any stray field may introduce an unexpected aberration or, if it is a fluctuating field, may blur the whole image.

To solve this complex of problems requires the greatest skill and ingenuity in designing the electron microscope, and the utmost care in testing it and tuning it up to top performance. From this viewpoint, the electron microscope is one of the finest precision instruments so far constructed by scientists and engineers. It is not surprising that it is correspondingly expensive. The final decisive stage of proving its performance is also one of some difficulty, as very few specimens are suitable for test purposes. They must possess micro-structure almost down to atomic dimensions, and be so thin that the electron beam can penetrate them with negligible scattering and energy loss. Deposits of metal, formed by evaporation from a hot filament in vacuum, are the easiest to make. Very thin crystals, in which particular planes are heavily loaded with atoms, are more difficult to prepare but provide a more convincing test, since the spacing of these planes will be known from X-ray diffraction experiments. The resulting test pictures are anything but exciting to look at, as in Plate 12c, but for the operator they are the decisive proof of the capabilities of his instrument.

The best results so far published are of the crystal lattice of gold, in which spacings of 2·04 Å and 2·35 Å have recently

been imaged (Plate 12c). In principle an instrument with such a performance should be able to resolve the individual atoms in a heavy metal such as gold or platinum, but the contrast given by a single atom is too small for it to be picked out on the photographic plate. A crystal lattice gives higher contrast, since it can be arranged with planes containing large numbers of atoms parallel to the electron beam, in the same way as we can look through a Venetian blind by getting the slats horizontal. Whether means can ever be found of seeing individual atoms in the normal type of electron microscope is still an open question, not least because an isolated atom is unlikely to stay stationary for very long. In any event, individual atoms are not very exciting things to study, any more than ball-bearings or glass beads. There is a wealth of fine structure in both the organic and inorganic kingdoms waiting to be unravelled by the electron microscope in its present state of performance.

What the Electron Microscope Reveals in the Biological World

The great strides which the electron microscope allows us to take, beyond the limit of the optical microscope, may be seen at once by comparing micrographs of one of the standard optical test specimens, a diatom. This is a small organism which lives in sea-water and grows a hard protective shell of calcium salts, containing regular holes. Plate 5A, B shows the increasing amount of detail in the light microscope as the aperture is increased to the maximum possible; a slight further improvement is provided by using ultra-violet light. The electron microscope then takes over and shows, not only the exact shape of the 'windows' but also that they are partly covered with a patterned net (Plate 5c). Incidentally, a diatom is useful as a low magnification standard for the electron microscope, since the dimensions of the main features of its structure are accurately known from years of observation with the optical microscope.

Bacteria form another interesting subject for comparison.

They were just visible as dots to Leeuwenhoek with his simple microscope, in bright illumination. The best light microscope is able to distinguish the main species by their shape—rod or spherical—and gives an estimate of their dimensions. The electron microscope reveals that they have considerable detail of form, and, from thin sections, a complicated internal structure (Plate 7A). It is also possible to identify and study the behaviour of a great variety of viruses which attack the bacterium itself, showing that the principle of 'Big fleas have little fleas . . .' extends a long way down the size scale.

The plant and animal viruses formed a natural subject of investigation for the electron microscope. They were known to exist from the diseases they caused, but only one or two of the very biggest could be seen in the optical microscope—the small-pox virus being one of these. Most of them were obviously very small indeed, since they would pass through the finest available filter. With the electron microscope their exact size and shape can be studied, and we are now discovering that many of them are not simple balls or rods, but have a complicated internal structure. The tobacco mosaic virus, which attacks leaves of the tobacco plant, was thought to be a typical rod virus but in fact it is a three-stranded helix, hollow in the centre. This structure is just visible in electron micrographs (Plate 9B), the fine details being filled in by X-ray methods. Many of the viruses which on first inspection seemed to be spherical have now been proved to be polyhedra, i.e. regular geometrical shapes with many plane faces, the simplest of which is the tetrahedron with four faces (a 'triangular pyramid'). Oddly enough the shape most frequently found is the icosahedron, of twenty faces. The polio virus seems to be of this form, but is rather too small to be sure about. The adeno-virus, which causes a throat infection, shows it more clearly (Plate 7B). The twenty faces are covered with protein particles, the number of which usually obeys the simple formula $N = 10(n-1)^2 + 2$, where n is a whole number, i.e. N is 12, 42, 92, etc. They form a hollow protective shell around the actual infectious unit, which is probably a thread of nucleo-protein. Some of the cancer viruses, and the warts virus, also are found to have this shape. How the living cell

contrives to manufacture such remarkably regular structures is still an intriguing mystery. The larger animal viruses are much more elaborate in structure. For instance, the influenza virus (Plate 7c) has an outer knobbly shell within which is coiled up, almost like a ball of wool, a very long chain or flexible rod.

A vast number of viruses have now been described in detail, but the problem of their nature remains open to argument. Are they living or inorganic, germs or molecules? They undoubtedly interact with living organisms so as to cause disease, but on the other hand they can be isolated like a chemical compound and some can even be crystallised, as shown in Plate 9a. A crystallised virus can be re-dissolved and is found to be still infectious. Discussion thus has to get down to an attempt to define the characteristics which distinguish living from non-living matter. One of these, the ability of self-reproduction, seems not to be possessed by viruses. They can multiply in an infected host cell, but not in a test-tube or on an inorganic culture medium. However, this failure may only be the result of our present ignorance of the reproductive needs of viruses, in the way of chemical compounds and general surroundings. So the classification of viruses as non-living can only be provisional. If the electron microscope was able to observe living material direct, we could follow what happens in an infected cell and perhaps get a true understanding of virus multiplication. But, in our existing instruments, the electron bombardment would quickly kill the cell, even if we could safely introduce it into the vacuum within the electron microscope.

Equally surprising has been the revelation of internal structure within the ordinary cells of plants and animals which the electron microscope has provided. From studies with the light microscope the cell was known to consist primarily of a nucleus surrounded by cytoplasm, with some evidence of other structures existing at least in certain stages of the life-cycle of the cell. But there was no suggestion of the complicated micro-architecture which the electron microscope soon showed to exist in the cytoplasm, and is now beginning to find also in the nucleus. Indeed, it has been necessary to coin the new

word 'ultra-structure' to describe it. It is, in effect, the macro-molecular scaffolding or framework within which, and perhaps on which, the dynamic processes of life proceed within the cell. Its investigation has been the chief contribution which the increased resolving power of the electron microscope has been able to make to biology.

Before this investigation became possible, however, it was necessary first to solve the purely technical problem of how to cut sections of tissue thin enough for electron microscopy. Electrons are very much less penetrating than X-rays or light, and even the thin sections a few microns thick, which are cut for optical microscopy, prevent a good electron image from being obtained. Generally speaking, we find that the specimen thickness should not be more than about ten times the resolving power we expect to achieve. So, to see detail of 20 Å, we ought to cut a section 200 Å thick. Initially there were not lacking pessimists who opined, and even claimed to 'prove', that it was impossible to cut sections thinner than about 1 micron. However, practical men soon showed that it was quite feasible to gear down the microtomes used for optical sectioning so as to cut 0·1 μ sections perfectly regularly. A microtome is similar to a bacon-slicing machine, but with a very fine screw or rocker mechanism to advance the sample by only a minute amount between each cut. To prevent tissue from breaking apart, it must either be frozen or embedded in wax or some similar solid medium. In the face of the demands of electron microscopy, much attention has been paid to refining all the stages of the process; finding harder embedding materials (often plastics), devising ultra-fine advance mechanisms, and investigating different types of knives. Carefully polished edges of diamond or sapphire are probably the best cutting means, but a sliver of plate glass is nearly as good and much cheaper, although it does not keep an edge for so long.

As a result, we now have available a number of ultra-microtomes capable of preparing sections as thin as 200 Å and, in favourable cases, even 100 Å. When one considers that these are only 100 atoms or less in thickness, the technical feat is quite remarkable. We have also found a way of preparing even thinner films of carbon (25–50 Å) on which to support

these sections in the electron microscope. Some impression of the increase in detail that is found may be gained from Plate 4, where optical and electron micrographs of the same subject are compared. Animal tissues are equally full of fine structure, as micrographs of liver, kidney or the pituitary gland show (see Plate 10A).

It is not too much to say that the new world thus opened up to the biologist is as rich in detail and as exciting to explore as that revealed when the compound optical microscope, with corrected lenses and high resolving power, first became available about 100 years ago. These new fields are being rapidly explored and the biological journals are full of micrographs mapping the micro-anatomy of the various organs and tissues of plants and animals. Indeed, it has been necessary to found several new periodicals to accommodate this new flood of knowledge. The next step in the advance is already being made: to identify the biochemical nature of these structures and to elucidate their function. Micro-chemistry and micro-physiology are following in the wake of micro-anatomy, in the same way as did the parent subjects.

A functional problem of particular interest is that of the motility of the larger micro-organisms, i.e. the means by which sperms, protozoa and related organisms propel themselves in water and other liquids. The electron microscope has shown that the 'tails' of all of them have basically the same structure, being made up of 2 central fibres surrounded by 9 finer fibres, and sometimes by a further outer ring of 9. The significance of this 'magic number' 9 is not yet clear, nor the difference (if any) in nature between the central and outer fibres. It is tempting to suppose that the central pair are nerve fibres which transmit signals all along the length of the tail to the outer ring of 9 or 18, which may be muscles for lashing the tail in particular rhythms. Whether this be so or not, sections of such flagella often show patterns of remarkable beauty (Plate 10B).

The Internal Structure of Metals

The discovery of ultra-structure in biological tissues and organisms was not entirely unexpected—although the wealth of it was surprising. It always seemed likely that there must be

units and structures within the large gap which separated the smallest details visible with the light microscope (about 0·1 μ) from the size of individual molecules (0·001 μ or smaller). But in metals the discovery of a great variety of micro-structures in this size range was much more of a surprise. A metal had been regarded as a regular arrangement of atoms, like a miniature climbing-frame with the atoms at the intersections—although other arrangements than a simple cubic one were possible. The dimensions and the main features of these 'crystal lattices' had been worked out by X-ray diffraction methods. True, it was known that most metals were not perfect crystals, and some theorists had maintained for a long time that there were many more defects within a metal than was commonly supposed, and that these had a profound effect on its strength and other properties of practical importance. The electron microscope quite early on showed some evidence of these 'dislocations', as they were called, but at first it was only possible to examine the surface of a metal, and this only by making an impression or replica of it in a plastic film. The breakthrough came about ten years ago, when metallurgists found ways of thinning metal strips down, in a controlled fashion, until they were thin enough for the electron beam to penetrate them, i.e. 200–2,000 Å thick. Such thin films are even thinner than the thinnest available gold or silver leaf, and indeed are transparent to light.

Appropriate micro-etching techniques have by now been found for practically all metals, alloys and semi-conductors, and so the electron microscope has become as important a tool for the metallurgist as for the biologist. Some of the remarkable features which occur within metals may be seen from Plate 12A, B. The general sub-division of a metal into grains and sub-grains, differing in the orientation of the lattice, was already well known. The new problems were the relationship of the various sorts of dislocations to the grain boundaries and to each other, and, in alloys, the relation between dislocations and precipitates (Plate 11). The new methods, indeed, provide a powerful tool for investigating the very earliest stages of precipitation, which are vitally related to problems of strength and fatigue.

As a further step, devices were made for carrying out experiments on the metal film whilst in the electron microscope. Micro-furnaces, cooling and straining stages were constructed—even chambers for carrying out chemical reactions by admitting gases. By photographing the viewing screen, and recently by building a ciné-camera into the microscope in place of the plate camera, motion pictures at any desired lapse rate can be taken of such processes. So the dynamic investigation of metallurgical processes has now been added to the other capabilities of the electron microscope (Plate 12B). In the Lectures two short films were shown on this subject, one of the spreading of a crack across an aluminium foil (made at the Royal Aircraft Experimental Station, Farnborough) and the other of the formation and re-solution of precipitates in an alloy steel as the temperature was changed (made at the Cavendish Laboratory, Cambridge). These techniques have recently been extended to semi-conductors and other non-metals, such as graphite, in which very similar dislocations are to be seen.

What next in Electron Microscopes?

Two main goals have still to be attained by electron microscopy: the imaging of individual atoms and the study of matter in the living state. As we have already seen, the route to the first of these goals is via the correction of spherical aberration and the control of various forms of instability in the microscope. Strenuous efforts in this direction are being made in a number of countries—in Germany, Japan, the United States, as well as England. There is every indication that the required resolving power of 2 Å will be achieved in the not too distant future (Plate 12C). It is less certain that it will allow us to image atoms visibly on the viewing screen or photographic plate, because of lack of contrast. A single atom would affect the electron beam so little that we might not be able to pick it out against the background—since the atoms of gold (or whatever we take) must be supported on a carbon film or something similar. One way of improving contrast is to reduce the electron voltage, and one or two scientists are exploring this

possibility. They have obtained quite good micrographs at a voltage as low as 5 kV, but it is too soon to say if the answer to the problem of resolving atoms really lies in this direction.

For the investigation of living matter, the trend is in the opposite direction—to higher and higher voltages. Two considerations are involved here. Firstly, we need greater penetration, because even a 100 kV beam will not go through the smallest living organism readily enough to produce a worth-while electron image. Secondly, it is found that the faster the electrons travel, the less is the damage they inflict on an organism of given thickness, contrary to what might be supposed. Roughly speaking, the chance of damage depends on the time an electron spends inside the organism, so the quicker it goes through the better. For these reasons, amongst others, French scientists have built an electron microscope designed to operate at a maximum voltage of 1·5 MV. The microscope itself is no more than an enlarged version of the standard type of instrument (see Plate 13); the main cost and complication goes into the equipment for obtaining the high voltage and keeping it sufficiently stable. Some interesting preliminary micrographs have been taken of various bacteria, maintained in their natural wet state in a special double-walled chamber. Although some of them survived the electron beam, it is not certain that they did not suffer serious damage in the process.

These experiments are still going on; if they finally succeed, they will open up a vast new field of study for the electron microscope, with the prospect of understanding the nature of living processes in greater detail than we now do. Meanwhile, the French high voltage microscope has also produced some impressive results from thick metal foils, many micrographs being of great beauty (Plate 14). This is an advance of considerable practical interest to metallurgists, since the ordinary electron microscope is limited to specimens not much thicker than 0·2 μ. It can fairly be objected that the structures that are visible in these films, after thinning them down from thick samples, may not be representative of the metal in bulk. Foils at least ten times thicker can be examined at 1 million volts and, although these are still far from the practical

dimensions of engineering, they are that much nearer to them. For this more prosaic reason, high-voltage microscopes are now also being built in England, Japan and elsewhere. It is not the first time that a project started with a particular aim in view has had a result of greater importance in an entirely different direction.

CHAPTER

5

POINT-PROJECTION MICROSCOPES FOR X-RAYS AND ELECTRONS

The Point-projection Principle

THE SIMPLEST WAY of making an enlarged image is to place an object in front of a point source of illumination, as you do when forming shadows on a wall with your hands in front of a candle or torch. A very big magnification can be obtained by bringing the object close up to the source, but the shadow will then begin to have blurred edges unless the source is very small. This is due to the half-shadow, or penumbra, where one side

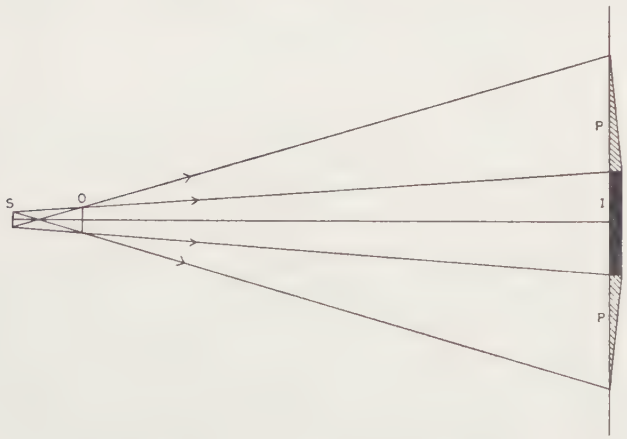

Fig. 37. Projection of an image of an object *O* by a point source of light (or X-rays) *S*. *I*=true image, *P*=penumbra.

of the source is throwing light into the shadow cast by the other part of it, as in Fig. 37. An object the same size as the source will form a poorly defined shadow, and a smaller object is unlikely to give a visible shadow at all.

So to make a microscope on this principle we need in the first place a source which is small compared with the object detail we wish to examine, i.e. a source smaller than the resolving power. It is also desirable to be able to bring the object very close up to the source, since the magnification is the image distance over the object distance (*SI*/*SO* in Fig. 37) and a very long throw (*SI*) will be needed for high magnifications unless *SO* is kept short. If we want to photograph the image, a short camera length also helps to keep down the exposure time because the illuminating intensity goes down, and the exposure goes up, with the square of the distance—the well-known inverse square law.

It is not very practicable to make a point-projection microscope of this type for light, partly because of the difficulty of producing a light source which is at the same time both very small and very bright, but also because such a microscope would be limited ultimately by the same diffraction effects as in the ordinary optical microscope. Since the latter has been brought to such a pitch of perfection there is no incentive for building the projection instrument. In the case of X-rays, however, the situation is quite different.

The X-ray Microscope

X-rays, as we saw in Chapter 1, are similar in nature to light rays, but are of very much shorter wavelength (Fig. 1). As the resolving power depends directly on the wavelength (p. 70), we would expect an X-ray microscope, if we could make one, to have a much better resolving power than the limit of the light microscope. The difficulty is that X-rays are refracted hardly at all by glass, or by any other material for that matter, so that practicable lenses cannot be made for focusing X-rays. The lenses used in the electron microscope also have no effect on them. Nor is it possible to make a mirror microscope, since X-rays pass right into the mirror instead of being reflected from it. The projection method thus provides the only possibility. The problem then is to produce a sufficiently small and intense source of X-rays.

The most straightforward way of producing X-rays is to

bombard a metal plate with fast electrons, as is done in the tubes used in hospitals for making X-ray photographs of broken bones. X-rays are emitted wherever electrons strike the metal, so to get a point source all we need to do is to focus an electron beam by the same methods as in the electron microscope. All we need, in fact, is the upper half of such a microscope, consisting of the electron gun and condenser lens system. Instead of directing an electron beam on to a specimen and obtaining an image by magnification in the objective and projector lenses, we direct the beam on to a metal foil which seals the end of the vacuum tube, as in Fig. 38. By adjustment of the magnetic electron lenses L_1 and L_2, the beam can be focused into a spot of any desired size down to a fraction of a micron. X-rays generated by the impact of the electron beam emerge from the foil in all directions, including forward into the air on the far side of it. If, now, a specimen is placed close to the foil, at S in Figure 38, its X-ray 'shadow' will be

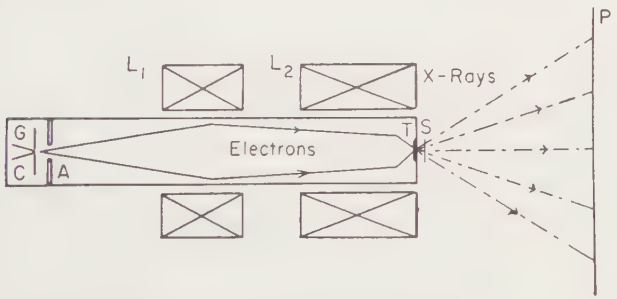

Fig. 38. Point-projection X-ray microscope, in outline. C, G, A = cathode, grid and anode of electron gun, L_1 L_2 = magnetic electron lenses, T = target of metal foil, S = specimen, P = photographic plate (or viewing screen). (*From* X-ray Microscopy *by V. E. Cosslett and W. C. Nixon; University Press, Cambridge.*)

cast on to a fluorescent screen at P. The fine detail in this image can be viewed with a lens or ordinary microscope, or it may be photographed by replacing the screen with a camera. It is helpful to have some reference mark on the screen, such as an inked cross, as otherwise it is hard to tell whether it is the X-ray microscope or the viewing microscope which is not in

focus. Focusing the X-ray microscope is carried out by changing the current in the magnetic lenses until the image on the screen is as sharp as possible, which means that the X-ray spot is at its smallest. The specimen itself need not be moved. It will be in focus wherever it is placed—only the magnification will differ.

The actual instrument is rather more complicated than appears from the diagram, because vacuum pumps and electrical supplies are required, although these are not so elaborate as for an electron microscope. The electron gun usually operates at a voltage between 5,000 and 15,000 volts, and currents of the order of an ampere or less are needed for the electron lenses. A complete X-ray microscope is illustrated in Plate 15, the column here being arranged vertically, with a strong viewing lens at the top instead of a compound optical microscope. For photography it is replaced by a simple plate camera. The specimen is mounted in a stage which allows motion in three dimensions: two traverses at right angles for searching it, and an axial shift for changing the magnification. If the camera is 10 cm. long, a convenient size, the magnification will be × 10 when the specimen is 1 cm. from the X-ray spot, × 100 when it is 1 mm. from it, and × 1,000 when it is as close as 0·1 mm., which is about the practical limit.

The exposure time depends on the intensity of the X-ray source, but it is always long compared with ordinary photography or even with electron microscopy—from 30 seconds to 30 minutes. The difficulty is to get enough current into a very small electron spot. Currents of hundreds of microamperes, or even milliamperes, could be drawn from the electron gun, but the spherical aberration of the electron lenses forces us to stop down the aperture of the final lens in order to prevent blurring of the focused spot. A few microamperes can be got into a 1-micron spot, but it is difficult to get even one-hundredth of a microampere into a spot of 0·1 micron. The X-ray output is correspondingly weak and exposure times long. A compensating factor, however, is that the heating effect of the electron beam on the metal foil is very low. So it is unnecessary to water-cool it, as has to be done for the target of a large medical X-ray tube, and it is rare for the

foil to be burnt-out unless the final aperture is accidentally omitted.

Resolving Power

The main limitation on the projection X-ray microscope is the intensity rather than the size of the source, as just explained. From the electron optical point of view there is no reason why the ultimate resolving power should not be as good as that of the electron microscope, a few Ångström units. But, with our present knowledge, the current obtainable in such a minute spot would be less than a micro-microampere and the X-ray output so low as to be invisible on even the best available screen. The practicable limit at the moment is about 0·1 micron (100 mμ), and even then the observer's eyes must be dark-adapted before he can see the image well enough for focusing.

To operate successfully with smaller spots will require one or more of three prospective developments: a more intense electron gun, the correction of spherical aberration of electron lenses, and an image intensifier for X-rays. Some improvement in electron guns is being made and there is hope for a corrected electron lens, but progress is very slow because of the complexity of the problem. In the immediate future the most promising line is the X-ray image intensifier. This is a type of electronic tube in which the image falls on a sensitive surface, as in a camera exposure meter, which emits electrons according to the intensity of the illumination. These electrons are accelerated and focused—and they may also be 'multiplied' at an intermediate surface—and finally fall on a viewing screen. If the tube is properly designed and operated, this electron image will have the same definition as the original image and be very much brighter.

Image intensifiers have been successfully produced for working with light, for use in astronomical telescopes, and with infra-red radiation for 'seeing' in the dark. The problem is more difficult with X-rays, but an intensifier has been made for medical purposes, so that for a chest X-ray (for instance) the patient need only be exposed to a low intensity. However,

these X-rays are much 'harder'—i.e. they are generated at much higher voltage—than those used in the X-ray microscope. Here we need the radiation to be only moderately penetrating, since we are examining small specimens and require a reasonable degree of absorption, or else there will be no contrast between thick and thin (or dense and less dense) parts of it. Some experimental intensifiers have been made for soft X-rays, and we may hope with their aid to be able to work with still smaller focal spots and weaker images than at present. But how far below the present resolving power of 0·1 micron this will enable us to go still remains to be explored.

Uses of the X-ray Microscope

Since the present resolving power of the projection X-ray microscope is no better than that of the ultra-violet microscope, the scope for its application is in the main to specimens where its peculiar properties give it an advantage over other methods. Metals and similar materials which are opaque to light offer a wide field, but so also do transparent specimens when they are so thick that a light microscope can show only part of them in good focus at one time.

As we saw in Chapter 3, the depth of focus of a compound microscope is not much more than its resolving power, so that when operating at high magnification to see fine details only a thin layer of a specimen is in focus, perhaps 1 micron thick or less. By sectioning the object we wish to study, a frog's muscle or an insect, for instance, we can get all the information we want with the aid of the light microscope, but it is a painstaking business. Drawings or photographs of each successive section have to be put together in order to understand the structure of the specimen in three dimensions, and it may even be necessary to build up a model from a series of wax or plastic layers corresponding to the sections examined. In the projection microscope, on the other hand, the whole of a specimen can be imaged at once so long as it will fit in between source and screen. The part nearest the source will naturally be magnified much more than that farthest from it, but will be almost equally sharp. By taking two successive photographs of the specimen

in slightly different positions, either tilted or shifted sideways a little, a stereo-pair of images is obtained. When these are put into a hand-viewer, or in a special type of projector, we get a three-dimensional view of the original specimen. In this way the structure of an insect, or of any thick object, may be revealed by two photographs instead of from a series of sections.

Viewing stereo-pictures requires a special pair of spectacles, and so their three-dimensional effect cannot be reproduced here. In the lectures several examples were shown, including the grain structure of an alloy and the distribution of graphite flakes in cast iron, as well as a spider's knee (Plate 17B) and various insects. Plate 16B shows a single X-ray micrograph of a fruit fly, illustrating the amount of detail and the effect of depth provided by the projection microscope. It has also been used for studying the internal structure of wood and of the leaves of plants, and has proved particularly useful for exploring the ramifications of the circulation of the blood. It was found, for instance, that the minute blood vessels beneath the skin on our finger-tips follow exactly the same fine pattern as appears on the surface. So if a criminal were to shave off the skin, to prevent his finger-prints being taken, an examination of the underlying blood vessels would nevertheless reveal them. It is even possible to study the changes in the circulation due to shock or drugs in animals such as the rabbit. Its ear is quite thin, once the hair has been shaved off, and can readily be X-rayed by laying the animal alongside the projection microscope. Plate 16A is a microradiograph in which the ramifications of the arteries into small capillaries, leading back into the veins, are clearly visible.

The X-ray projection microscope is also finding uses for investigating the internal structure of inorganic materials such as metals and plastics. It helps to supplement the information provided by the electron microscope, which can deal only with very thin specimens, as we saw in the previous chapter. Plate 18B is an example, showing the way the tin (dense regions) is distributed in an aluminium-tin alloy. Similarly one can make visible the dispersion of fissile material in the fuel element of an atomic reactor, or of empty spaces within a porous substance such as carbon. In general the

method is useful for revealing any sort of inclusion, precipitate, vacant space or non-uniformity so long as it has a different absorption power for X-rays from the surroundings. Roofing materials, mineral ores and foamed plastics are a miscellaneous group of examples. The fine structure in plastic beads is especially striking (Plate 18A).

The fact that contrast in the X-ray image is due mainly to differences in absorption makes it possible to identify particular chemical elements. The absorption coefficient, which is a measure of the amount by which a layer of material weakens the X-ray beam, depends on the nature of the layer, i.e. on the chemical elements in it. For a particular element the absorption coefficient increases rapidly as the wavelength increases, but the rise is broken by sharp drops from time to time, called the 'absorption edges'. The position of the edge varies from element to element in a regular manner, so that two different elements will have them at quite different wavelengths, as shown in Fig. 39. So if we make measurements of the X-ray intensity transmitted through a specimen at two wavelengths, one on each side of an absorption edge, the difference will be due almost entirely to the presence of that particular element, and by comparison with test experiments on known thicknesses, we can calculate how much of it is present in the specimen.

In this way the projection X-ray microscope provides a means of analysing quantitatively for any chemical element likely to occur in a solid substance, and it is not affected by the element being part of a compound. It is only necessary to provide an attachment for selecting the particular wavelengths at which absorption measurements have to be made. This functions by reflecting the X-rays from the surface of a crystal and is usually known as the Bragg spectrometer, as it was originally devised by Sir William Bragg, a former Director of the Royal Institution, and his son, Sir Lawrence Bragg, the present Director. We shall have more to say about its construction and uses in the next Chapter.

This micro-absorption method allows us to explore the variation in amount of an element in different parts of a specimen. A general picture of the distribution is provided by taking X-ray photographs of it with two different wavelengths,

one on each side of the absorption edge of that element, and comparing their appearance. Regions which contain the element will be dark in the picture taken at the wavelength for which the absorption coefficient is high and much whiter in

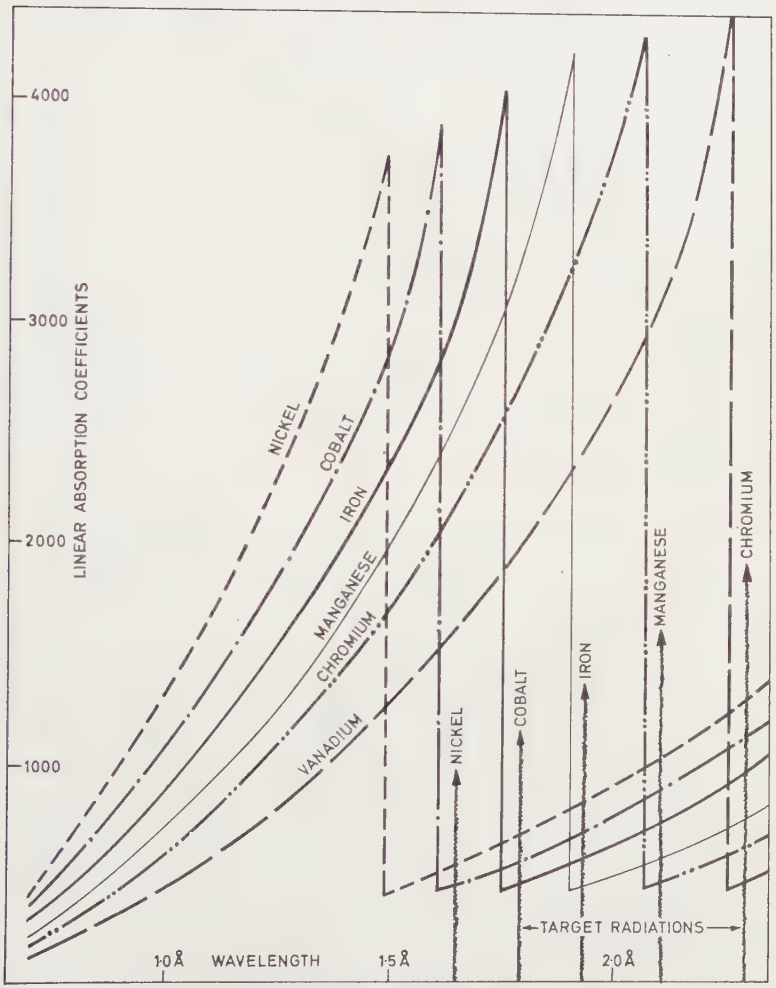

Fig. 39. X-ray absorption edges for neighbouring elements in the periodic classification. The absorption coefficient at first increases as X-ray wavelength increases, then falls sharply at a wavelength just short of the characteristic X-ray wavelength emitted by a target of the same element. (*From R. S. Sharpe, in* X-ray Microscopy and Microradiography; *Academic Press, New York and London.*)

that at the low absorption wavelength. Plate 17A shows an example, in which the titanium present in an ore can be identified in a pair of radiographs taken at two specially selected wavelengths. In another study, the distribution of calcium in monkey's teeth was mapped out by direct analysis with a projection microscope fitted with a Bragg spectrometer. A minute aperture was placed over each selected point of a tooth section in turn, and the intensity of the X-rays transmitted at wavelengths on either side of the calcium absorption edge was measured with a Geiger counter. By this method it is possible to follow the changes in calcium content at various ages, and also to detect differences between teeth from different sites in the jaw or between healthy and diseased teeth. However, it is a rather time-consuming procedure, since measurements have to be made at two wavelengths for each point. For this reason the X-ray projection microscope is mainly used for survey studies, quantitative analysis being more readily carried out by the X-ray emission technique described in the next Chapter.

The Field-emission Microscope for Electrons

It is perfectly feasible to make a point-projection microscope for electrons on the same lines as for X-rays. We need only remove the metal foil from the end of the tube of Fig. 38 and allow the electron beam to fall directly on the specimen. An electron shadow would then be projected on to the screen, which now would have to be in vacuum also. This type of electron microscope has been constructed, but it proves to have no particular advantages over the ordinary electron microscope or the X-ray projection microscope.

A more interesting and useful development has been the point emission electron microscope, in which the structure of a metal tip is imaged at very high magnification by means of the electrons emitted from it. The principle is extremely simple: if a strong electric field is applied near the surface of a metal, electrons are dragged out and accelerated away from it in

straight lines. To produce appreciable emission the field must be higher than about ten million volts per centimetre, but it would be quite impracticable to set up a voltage anything approaching 10,000,000 volts at 1 cm. distance from a flat metal plate. However, a much lower voltage is effective when applied in a particular manner, which relies on the same physical principle as the lightning conductor. When a high voltage is set up near any surface, the electric field is most concentrated around curved projections and especially at sharp points. The smaller the radius of curvature the greater is the field, and in good approximation the field is in fact given by the applied voltage divided by the radius of the point. By making a very minute needle point, we can produce an enormous electric field around it with the aid of a quite moderate voltage. With a radius of 1 micron, 10 kilovolts will give a field of $10^4/10^{-4}=10^8$ or one hundred million volts per cm. Under such a field, electrons would be emitted so copiously that an electric arc would probably start and the tip would be destroyed.

This type of emission is usually termed 'field emission' from the way it is generated, or sometimes 'cold emission' in contrast to thermionic emission, which results when a filament is heated. The fine tips are made by etching a thin metal wire under carefully controlled conditions. Tungsten, which was the material first used, can be etched either chemically in sodium nitrite or electrolytically. With practice it is possible to obtain tips with a radius smaller than 0·1 micron, i.e. a few hundred Ångström units, in which there are only a few hundred atoms across the diameter. These atoms are arranged in a regular lattice, since tungsten like any other metal is a crystalline substance. The electric field will be most highly concentrated around any projections on the surface, such as are bound to occur at the edges or corners of a cubic lattice. The electron emission will therefore be most intense from these projections, and so the pattern of brightness in the emitted beams will reproduce these features of the crystal structure. The electron image on a fluorescent screen will be a projection of the arrangement of the atoms in the tungsten tip.

The simple arrangement which enables this to be done is

called a 'field-emission microscope', and is shown in Fig. 40. The metal tip, carried on a supporting wire, is fixed at the centre of a glass bulb. The forward half of the bulb is coated inside with fluorescent material, and in front of it is mounted a metal ring. The high voltage, of the order of a few thousand

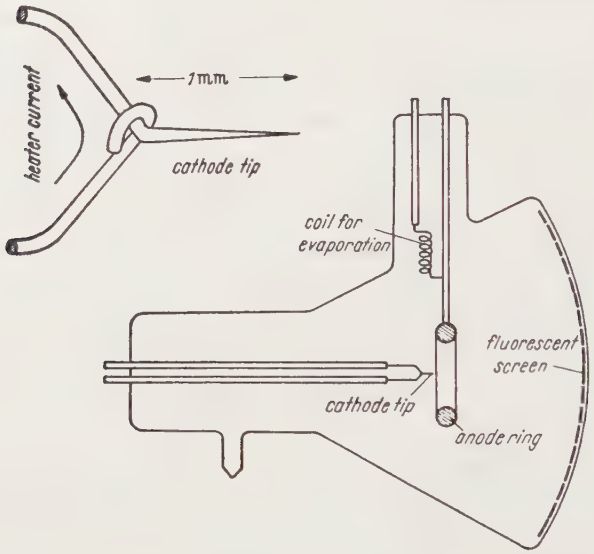

Fig. 40. Field-emission electron microscope: schematic cross-section. (*From R. H. Good and E. W. Müller, in* Handbuch der Physik, *Vol. 21; Springer, Berlin.*)

volts, is applied to this ring which thus acts as the anode, the tip being the cathode. When the voltage is switched on electrons are extracted from the tip and a bright image appears on the screen as the voltage is raised. If the image is to be a proper representation of the tip, the vacuum in the bulb must be extremely high—much better than in an ordinary electron microscope and even better than that in a television tube or electric light bulb. Otherwise the tip will quickly be 'contaminated' by oxygen, nitrogen or other molecules in the residual gas inside the bulb. To attain this ultra-high vacuum the whole bulb, complete with tip and screen, is 'baked-out' by heating in a furnace whilst being evacuated with special pumps. On the other hand interesting experiments can be carried out

by deliberately introducing atoms or molecules of known substances in controlled amounts, which may cause marked changes in the image and thus show how they are absorbed on particular facets of the tip. The small coil shown in Fig. 40 is used for this purpose.

A typical electron emission image from tungsten is shown in Plate 19A. It shows that some parts of the tip emit much more brightly than others, in a well-defined pattern which can be related to its crystal structure. When certain foreign atoms such as barium, are admitted to the bulb they will settle on the tip in positions where the force of attraction is greatest. They stay on the surface and are said to be 'adsorbed', and fortunately they enhance the emission instead of reducing it as does oxygen or carbon. Consequently the electron emission image shows up the pattern of adsorption very clearly, as in Plate 19B. Studies of this type are important in connection with the formation and properties of the oxide-coated cathodes generally used in radio valves, television tubes, etc., in preference to tungsten filaments.

Other metals besides tungsten can be examined in the field-emission microscope, but the choice is limited since they must be capable of being prepared as very fine tips and of standing up to the relatively large current density drawn from them. Although the maximum current may only be 100 microamperes or so, it comes from such a minute tip that the *current density* is very high, and so the heating effect is great. The area of choice is thus limited to metals of high melting point, such as tantalum, rhenium, platinum and molybdenum.

Ideally, if the electrons were projected from the tip exactly in straight lines and if the magnification was high enough, the individual emitters would be imaged and we should be able to distinguish the position of single atoms. In practice the electrons have a slight sideways velocity when emitted and they are also diffracted in the surface field owing to their wave nature, with the result that the resolving power is at best 10–15 Å (1–1·5 mμ). The magnification is given by the ratio of the radius of the bulb on which the screen is carried to the radius of the tip. So, if the former is 10 cm. and the latter 0·1 micron, a direct magnification of 1 million times is obtainable,

far and away the highest value in any type of microscope. With this magnification an emitting spot of 1 mμ diameter will be imaged at a width of 1 mm., and so be clearly visible on the viewing screen. Very little can be done about the factors which set a limit to the resolving power of the field-electron microscope, and it is unlikely ever to do better than about 10 Å.

The Field-ion Microscope: Imaging the Atom

An appreciable improvement is available, however, by using ions of a suitable gas instead of electrons for the imaging process. This is done by admitting a very small amount of one of the inert gases—helium in the first place—to the glass bulb and reversing the electrical connections, so that the tip is now the positive anode and the screen is the cathode, as in Fig. 41. When a neutral atom drifts up to the tip it becomes ionised: an electron is dragged out of it in the strong electric field, and, being now a positive ion He^+, it is repelled at high speed from the positively charged tip. The ionising effect is strongest where the field is highest, i.e. at the sharpest projections from the surface, as in the field-electron microscope, so that an 'ion-image' of them is formed on the fluorescent screen.

The essential difference from the field-electron microscope is that the helium atom is some 7,400 times more massive than the electron. So it receives less sideways velocity when repelled from the surface, and also it is diffracted less, since its equivalent wavelength is much smaller. To keep the lateral velocity as small as possible the tip is cooled down to the temperature of liquid hydrogen. The system is thus considerably more complicated than the field-electron microscope, as Fig. 41 shows. On the other hand the ion currents are much smaller ($10^{-8} - 10^{-10}$ amps) than the electron currents of the latter, and the image on the screen is correspondingly fainter. Sometimes the operator has to work metaphorically as well as literally in the dark, taking long-exposure photographs (up to 1 hour) of the screen to see what the details of the pattern are like. In recent times suitable image intensifiers have become

available, of the type mentioned in connection with the X-ray projection microscope, and it is now possible to operate the field-ion microscope with reasonable visibility of the image.

All this complexity of construction and operation is worth while for the good reason that the end result is a higher practical

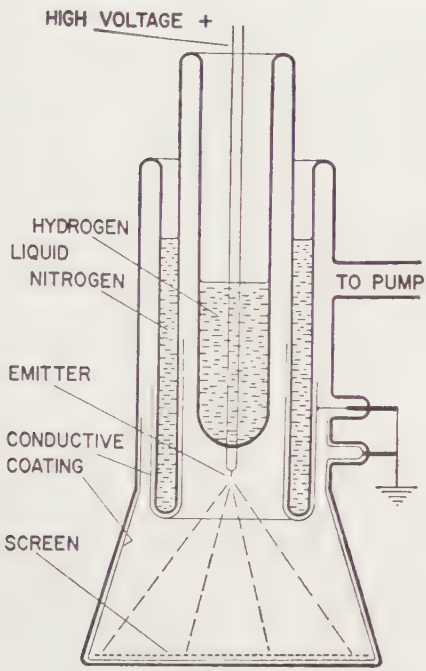

Fig. 41. Field-ion emisson microscope, operated at very low temperature: schematic cross-section. (*From E. W. Müller, in* Advances in Electronics and Electron Physics, *Vol. 13; Academic Press, New York and London.*)

resolving power than that of any other microscope. The field-ion microscope is unique in making atoms, or more precisely the sites of atoms, directly visible. Plate 20 shows the pattern obtained from a tungsten tip with a currrent of 10^{-9} amp. of helium ions. Higher voltages are used than in field-electron microscopy, and in this case 21,300 volts were applied. The image is brightest in concentric rings around certain dark regions. These latter are flat facets of the metal tip, and some of them have been labelled with the appropriate crystallographic indexes. Being flat, the field is less strong over these regions and so there is little or no emission from them. But the field will be very strong at the edges of the atomic layers which build

up these projections, like terraces, and the consequent high emission causes the bright rings. In many rings the individual atoms can be seen well separated from each other; the magnification is known, and so we can be sure that these are single atoms and not groups.

The atomic arrangements in tips of many different metals have been made visible by this technique: iridium, molybdenum, rhenium, rhodium, tantalum, tungsten, as well as platinum. The information obtained is not new, because the crystal structure of all these metals had already been discovered by means of X-ray diffraction. What is new, however, is that any defects in the regular lattice of atoms at the surface will now show up in the field-ion image. Further, by increasing the electric field strength to a very high value for a short time, it is possible to strip off a layer of atoms from the surface, revealing the next layer in the lattice. This 'field-evaporation' technique demands very careful control of the conditions, but is proving most valuable in enabling us to explore below the surface of a tip.

As mentioned in connection with electron microscopy, the extremely minute defects which occur in a metal can have a great effect on its mechanical strength and other properties. The electron microscope makes visible dislocations and faults composed of a group of atoms, but its resolving power is not yet high enough to show where an individual atom is missing or some foreign atom has been trapped in the crystal lattice. The field-ion microscope now gives us the exciting possibility of doing just this, and great efforts have been made to extend its scope to include metals of more everyday interest to the metallurgist, such as iron and nickel. They do not give very good images with helium ions, but fortunately the results are much better when neon is used. For copper and titanium, argon has been successfully used.

The field-ion microscope thus provides a most powerful tool for studying the various types of crystal imperfection with a resolving power that makes individual atoms (or their absence) visible. In some cases the defect can be produced during observation of the screen. As a microscope it is rather complicated and requires skilled operation, but nevertheless it is

now being increasingly employed in metallurgical laboratories for research on practical problems. These include chemical reactions at the surface and, perhaps more important still, radiation damage produced (for instance) by exposing the metal tip to fissile particles in a nuclear reactor. Plate 19c is an example, showing how clearly the disruption of the atomic lattice is imaged. In spite of being limited to very minute samples of a metal, the field-ion microscope is providing information of great value which could not be obtained by any other means. After many years of development it is now at last justifying the faith and labours of a few pioneer scientists in Germany and America.

CHAPTER

6

FLYING-SPOT MICROSCOPES

In the microscopes we have so far considered, the image is formed as a whole, that is to say, an illuminating beam falls on all of a given area of the specimen at the same time and the whole image is recorded in a single exposure. This is the procedure in both the compound microscope and the point-projection system, no matter whether light, electrons or X-rays constitute the illumination. We shall now consider an entirely different method of microscopy in which the image is built up point by point or line by line, in much the same way as a newspaper picture is composed of a great number of minute dots of varying strengths. Line by line or 'sequential' imaging is familiar to us nowadays in television. The optical image which is projected by a lens into the television camera, is scanned line by line with an electron spot. The electrical signal thus generated is picked up in the receiving set and used to modulate a similar electron spot scanning across the viewing screen, i.e. to vary the strength of the fluorescence it produces along each line of the scan. In this way, the variations in brightness of the original optical image are reproduced in the image seen by the viewer on his set.

In television the final image is nearly always smaller than the actual scene, but it is a straightforward matter to adapt the scanning principle for microscopy. The resulting system is then usually called a 'flying-spot' microscope, to distinguish it from a 'television microscope' made by simply fitting a television camera on to the eyepiece of a light microscope, or in front of the viewing screen of an electron microscope. In the flying-spot system (Fig. 42) a fine spot of light or electrons, as the case may be, travels across the specimen in a regular pattern and the

amount of illumination passing through each successive point it covers is recorded by a photo-cell or a counter. The resulting electrical impulse, the 'signal', is amplified electronically and fed to a cathode-ray tube in such a way as to produce an

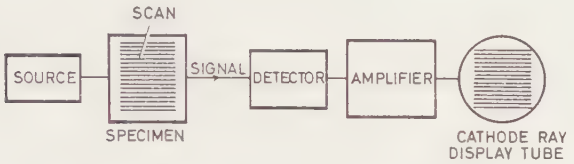

Fig. 42. Block diagram of the flying-spot microscope system.

image on its screen. This picture will reproduce the specimen in terms of its transparency for light (or electrons), absorbing regions appearing dark and vice versa. Illumination reflected from the specimen surface can also provide the signal, allowing us to study solid objects.

The magnification is given by the ratio of the width of the scan on the display tube to the width of the original scan on the specimen. It is convenient to keep the final image at constant size and so the magnification is changed by varying the area scanned on the specimen. The resolving power will be limited in the first place by the size of the flying spot and, as in the X-ray projection microscope, the operating conditions are usually arranged so that other factors have a lesser effect on the image. The ultimate limits to performance are then set by the minimum size of spot which can be formed with adequate intensity, and on the other hand by the sensitivity of the detection system. These factors, and so the performance of the microscope as a whole, will vary according to the type of illumination used.

The flying-spot microscope was first made with visible light as illumination, and later on for ultra-violet radiation. Then the principle was adapted for electrons, where it has provided a means of investigating surface structure, supplementing the transmission electron microscope. More recently still, the X-rays generated by a flying electron spot striking the specimen have been utilised as signal, since they can be detected by a suitable counter. This X-ray microscope has in fact proved

to have many more practical uses than the light or electron flying-spot microscopes, mainly because the X-rays give direct information about the chemical elements present in the specimen. For this reason the instrument has come to be known as the 'electron-probe microanalyser' rather than by its original title, the 'scanning X-ray microscope'. It is now being widely used for analysing the microscopical constitution of metals and minerals, and to some extent also biological specimens.

The Flying-spot Light Microscope for Particle Counting

Since we cannot deflect light rays so simply as we can an electron beam, the scan in the flying-spot light microscope has to be produced in some other way. The method usually adopted is, in fact, to make use of an electron scan: the pattern of the spot on the screen of a cathode-ray tube is demagnified and projected on to the specimen. To do this, an ordinary microscope is used backwards as in Fig. 43, the light passing through the eye-piece and the objective on to the specimen. The amount of illumination passing through it at any position of the spot is recorded by a photocell or photo-multiplier, the current from which is amplified and passed to the display tube. By appropriate control of the amplifier it is possible to increase artificially the contrast in the image of a poor specimen.

The instrument as a whole is understandably much more complicated than a normal optical microscope, and it is correspondingly expensive. A complete installation is shown in Plate 21. The cost and complexity is justified by its capabilities, since it can do things which are outside the scope of the ordinary instrument. In particular it can measure the size of small particles, and this is one of its chief uses. Whenever the flying spot crosses an opaque area of the specimen, the light to the photocell is cut off and the signal falls to zero. The length of time for which this occurs is a measure of the size of the opaque region, and this can be detected by an accurate timing mechanism in the recording system. It would be

possible in this way to get a measurement of every particle in a specimen composed of a sample of dust, for instance, but the problem is how to handle the results without slowing down the spot to a very low speed.

The answer is provided by a sizing procedure which automatically sorts out the measurements and grades the particles

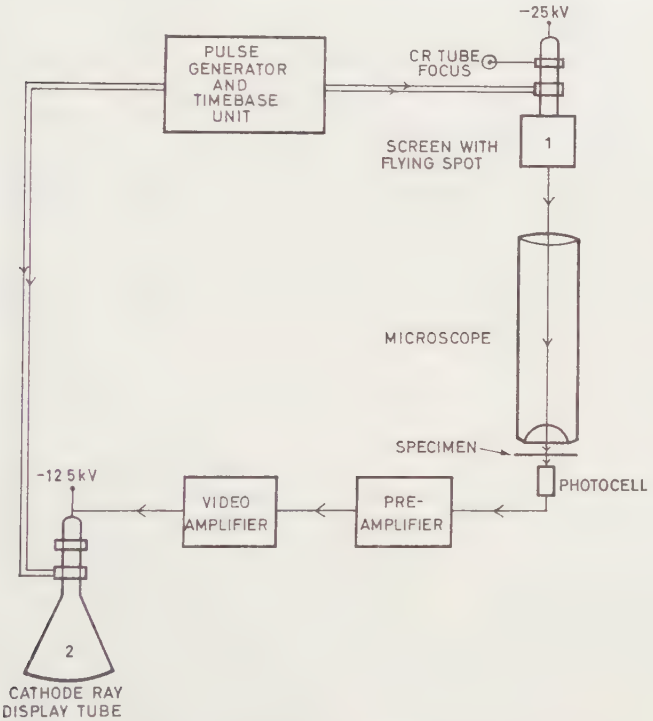

Fig. 43. Outline diagram of a flying-spot optical microscope. The flying spot of light on the screen of cathode ray tube (1) is demagnified by the microscope and scans across the specimen. The varying amount of light transmitted through it, as recorded by a photocell, controls the brightness of the spot on the screen of cathode ray tube (2), thus building up an image.

according to size. A timing mechanism is arranged to record only those interruptions of the signal which last more than a given length of time, so that it 'counts' only particles greater than the corresponding size, as given by the speed of travel of

the flying spot. To avoid counting any particles more than once, the mechanism is adjusted electronically not to respond again to a zero signal from that part of the scan until after a positive signal has been received, indicating that the spot has cleared the particle. In other words, the recording system has a 'memory' for each particle after it encounters it for the first time. Nevertheless a double count will not be avoided when a particle has an indentation, like a figure of eight, or a count may be missed when two particles overlap, but a statistical correction can be made for these possible errors.

The recording system gives in this way a series of totals for the number of particles which exceed successively larger sizes, increased in steps by varying the response time of the mechanism (Fig. 44*a*). By difference we obtain the number of particles in each size range, and in many instruments this differencing is done automatically. The end result is a chart or histogram showing the frequency of occurrence of each

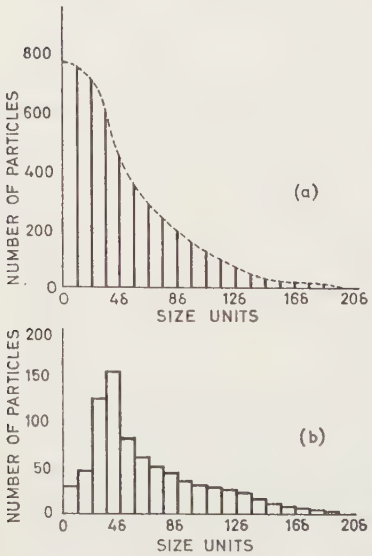

Fig. 44. Typical output from a flying-spot optical microscope programmed for sizing particles. (*a*) is a record of the number of particles larger than a given size, as marked on the horizontal axis. (*b*) is a chart ('histogram') of the number of particles in each size-range, obtained by subtracting each number recorded in (*a*) from the preceding number. (*From W. K. Taylor, in* British Journal of Applied Physics, *London, Vol. 5.*)

different size of particles, as in Fig. 44*b*. Alternatively the microscope can be fitted with an electronic integrator in order to measure the total area of particles in the field of view instead of their number.

The flying-spot optical microscope is thus as much a computer as a microscope. It finds uses wherever large numbers of similar particles have to be graded. They may be mine dusts, which have to be monitored in the interests of preventive health, or blood cells, in connection with diagnosis of active disease. Another biological use was for counting the number of nerve fibres in a section of a brain. Other applications include the sizing of pigment particles for use in paints. To carry out this sort of analysis by direct observation would require so many individuals peering through ordinary microscopes for such long hours as to be quite impracticable. The flying-spot instrument can cope with particles at the rate of several hundred per minute, and never gets tired. It has opened up methods of continuous control of processes and patients which previously were out of the question.

Ultra-violet illumination can be used, in place of visible light, by coating the screen of the first cathode-ray tube with a material which emits the required irradiation when bombarded by the scanning electron beam. This gives rise to some practical difficulties, however, as the wall of the tube must now be made of a special type of glass or of quartz in order to be transparent to ultra-violet rays. An alternative method is to reflect a beam of the radiation from a mechanically driven scanning system comprising rotating or vibrating mirrors. The ultra-violet flying-spot microscope is especially valuable because the damage caused to a sensitive specimen, such as a living cell, is much smaller than it is in an ultra-violet microscope of normal construction, with continuous whole-field illumination.

Ultra-violet microscopy has an advantage over visible light in that it can detect the presence of certain vital constituents of living matter, the nucleic acids, but at the same time these are particularly sensitive to radiation damage. For this reason the flying-spot principle has considerably extended the scope of ultra-violet investigations. It has been used in cancer research, for instance, to measure the amount of nucleic acid in mouse tumour cells in different stages of development.

The Electron Scanning Microscope

The basis of an electron scanning microscope already exists in the projection X-ray microscope of Fig. 38. It is only necessary to supply a means of deflecting the electron spot in the desired regular scan and to replace the metal foil target with the specimen itself. The remaining need is for some detector of the electrons transmitted through it, and this can be a Geiger or some other type of counter. This type of flying-spot microscope was thought of before the Second World War, but only became a practicable instrument after the War when sufficiently sensitive detectors had been developed. Since then its possibilities have been thoroughly explored, and found to be more promising for the microscopy of surfaces than for transmission.

The main features of a practical electron scanning microscope can be seen in Fig. 45. The electron gun is similar to that of a

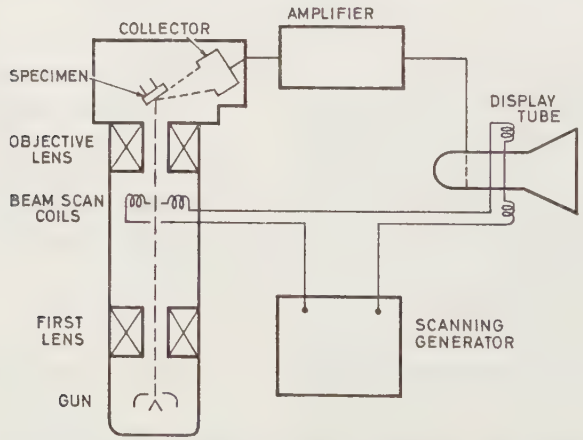

Fig. 45. Outline diagram of the electron scanning microscope. (*From K. C. A. Smith*, in Pulp and Paper Magazine of Canada, *Vol. 60.*)

normal electron microscope, but the accelerating voltage is lower, being adjustable from 5 kV to 25 kV. The first condenser lens is weak and the second is strong, as in the X-ray projection microscope. Between the first and second con-

denser lenses a set of deflecting coils is placed for scanning the electron spot across the specimen, in the same way as is done in a television tube; alternatively pairs of plates can be used, to which an alternating voltage is applied. The specimen is situated just below the second electron lens, with its surface at an angle to the direction of the scanning beam. The electrons which bounce back from the surface are attracted by a positive voltage on to the collector, which is usually a scintillation type of counter. This is a small block of a substance, such as sodium iodide, which emits a pulse of visible light when struck by an electron. The amount of light emitted is thus a measure of the number of electrons received. The light is conducted by a 'light-pipe' of perspex to a photo-multiplier which produces an output current proportional to the light input. This current is further amplified and fed to a cathode-ray display tube, as in the other types of flying-spot microscope. The variations in brightness of the displayed image correspond to the differing 'electron reflection power' of different parts of the region scanned on the specimen. The amount reflected depends on the local composition and the relief of the surface—parts tilted towards the collector will contribute more electrons than those facing away from it and so will appear brighter.

The scanning electron microscope is thus considerably more complicated than the ordinary type, and demands greater skill to operate it. Its design is made particularly difficult because the electron signal from the specimen is very small. The electron spot may be only a few hundred Ångström units in diameter, carrying a current of no more than a micro-microampere (10^{-12} amp.). No amount of amplification, however great, will produce an intelligible image if the input signal is too weak. One way around the difficulty is to use a very slow scan, so that the spot stays on a given part of the surface for a longer time than in a normal scan and so can contribute a larger number of electrons to the collector. A repetition rate of 1 second or more for a complete scan (one 'frame') may be used, instead of the 25 frames per second of normal television practice; the number of lines in a frame can be varied from 150 to 400, depending on the image resolution required.

The need for a slow-speed scan makes the design of the amplifier and deflection system more tricky than at higher speeds. It also makes it necessary to use a special type of cathode-ray tube for the display, of the long-persistence type, in which the fluorescence does not fade so quickly as in the usual television tube. If the decay time to half-brightness is 20–30 seconds, as it is for some phosphor materials, successive frame-scans of 1 second duration will reinforce each other so as to form a much brighter image. Even so it is sometimes possible for the eye to see the separate lines making up the image. When it is photographed a still better image is obtained, as exposures of 1 to 5 minutes are usual, taking in many frames.

The resolving power of this type of microscope is limited, as in the X-ray projection microscope, by the size of the electron spot and the amount of current that can be focused into it. The reflection of electrons from a surface is much more plentiful than the production of X-rays, and so a considerably smaller beam current can be employed, even down to 10^{-13} amp. With our existing types of electron lenses such a current can be obtained in a spot rather smaller than 100 Ångström units (10 mμ) in diameter. There is in any case little point in making a spot much smaller than this, because the electrons spread as they enter the surface and thus enlarge the region from which reflected (or 'secondary') electrons are contributed to the collector. The best resolving power so far attained is about 100 Å, more than ten times poorer than that of the ordinary transmission electron microscope. But there is some hope that, with a radically different type of electron lens now being developed, the performance of the latter instrument will be more closely approached in the future. To do so will also require lowering of the voltage of the electron beam, to reduce its penetration into the specimen.

The great advantage of the scanning microscope is, of course, its ability to examine the surface of solid specimens. It is possible to adapt the transmission electron microscope to image a surface by reflecting the whole electron beam from it into a normal objective lens, but the total current on the specimen is then large enough to cause damage to all but the most resistant materials. In the scanning system the beam current is much

smaller, and also the surface is only exposed to it intermittently during the scan. So quite sensitive specimens, such as fibres or plastics, can be investigated as well as metals.

The main value of the scanning microscope is for examining the roughness of surfaces. The depth of focus of the image is remarkably large, and often gives a three-dimensional appearance. One use is to study the way in which a material fractures, as revealed by the form of the resulting surface; both metals and ceramics give interesting results. Similarly surface wear during polishing and grinding can be followed, or the process of etching. A recent development has been to observe the progressive removal of a metal under bombardment by a beam of argon ions, produced by an ion gun (similar to an electron gun) built on to the final lens of the scanning system. The results show that some parts of the surface are removed much faster than others, leaving cliffs and valleys, or pinnacles as in Plate 22.

The scanning microscope has been much used for examining the outer form of fibres, both natural such as wool and artificial such as nylon. They are much too thick to be penetrated by the beam of an electron microscope, and even to come within the depth of focus of the light microscope, but their scanning images reveal a great deal of detail. The same is true of any round object, such as a needle (Plate 23A), or even of the surface of wood (Plate 23B) and of paper, which consists of a mass of compressed cellulose fibres. All such specimens have to be coated with a thin layer of aluminium to render them conducting, since otherwise they would charge up irregularly in the electron beam and give a very confused image. In the same way the surface structure of a variety of biological specimens has been examined, such as the cuticle of insects, the gut of a rat and the dentine of teeth.

An interesting example of a very sensitive specimen is the type of explosive, such as silver azide, which is detonated by very slight heating and so cannot be examined in the normal electron microscope. It was possible to mount the needle-like crystals in the scanning microscope in such a way that their progressive decomposition on mild heating could be clearly followed.

So far we have been dealing with specimens in which the

variations in image brightness are mainly due to surface relief, which throws differing amounts of the electron beam into the collector. But the reflection of electrons will also be affected by small differences in voltage between different parts of the surface. Such differences occur naturally at a boundary between two different metals, and this 'contact potential difference' shows up very clearly in the displayed image as the electron beam scans across it. A special case of this type occurs in a transistor. The potential differences are only a fraction of a volt, and yet they are readily visible, as in the scanning images of Plate 24. It was recently discovered that the results are even more informative if the image is formed by the current flowing into the specimen when the beam scans it, instead of by the reflected current. In this form the scanning electron microscope is proving of such value for the study of transistors made by diffusing one metal into another that several commercial models of it are now being produced.

It is perhaps surprising that this type of microscope is now finding such very different applications—on the one hand for transistors and on the other hand for natural and artificial fibres—at a resolving power which is not much better than the limit of the optical microscope, and at a cost which is twenty times greater. The production models at the moment have a resolving power little better than 0·05 μ (500 Å), and only one or two research instruments have attained 100 Å. The merit of the technique, of course, lies in its ability to examine surface structure and to detect differences of potential, neither of which can be done by any other method of microscopy. Even if the resolving power cannot be pushed much farther—and opinion on this point is equally divided between the pessimists and the optimists—it is assured of a continuing role in its present form. Indeed, if it should find uses in routine inspection of industrial products, whether fibres or transistors, it may become more widely used than the ordinary electron microscope. There are also signs that it may be valuable for examining the microcircuits and similar details involved in the miniaturisation of radio and other instruments in connection with space exploration.

The X-ray Scanning Microscope or Microanalyser

In the electron scanning microscope the moving spot causes X-rays to be emitted from each point of the specimen over which it passes. If these X-rays are detected, instead of the reflected electrons, we have an X-ray microscope in which the constrast variations in the image will depend on the amount and nature of the X-rays emitted from different parts of the specimen. The advantage of doing so is that X-rays are very characteristic of the substance which emits them. Each chemical element emits only a few well-defined wavelengths, usually well separated from each other, as indicated in Fig. 46, and known as X-ray 'lines'. So if we have available a

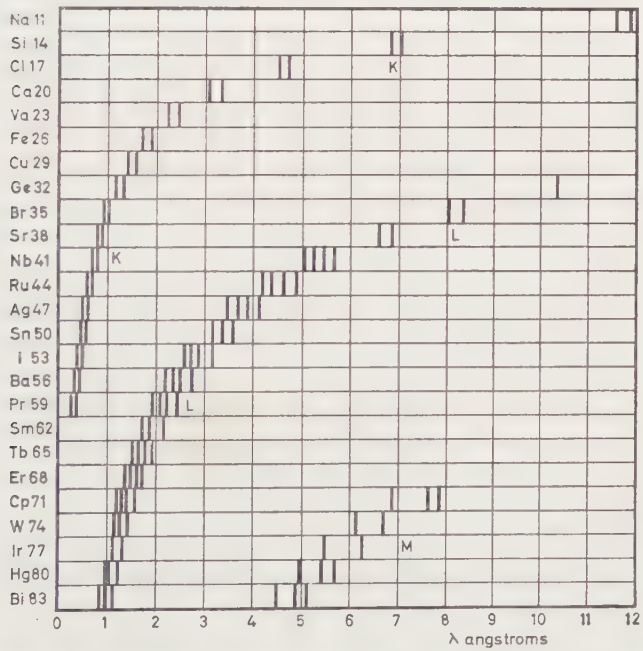

Fig. 46. Chart of the wavelengths of the characteristic X-ray lines emitted by the elements. In the K series each element has two strong lines only, in the L series several lines at longer wavelengths, and in the M series (at still longer wavelengths) a large number of which only a few are shown here.

collecting system which will detect particular X-ray lines, and record the intensity of each, we have a method for not only identifying the elements in a specimen but also analysing the amount of each present.

The X-ray scanning microscope is thus a simple development from the electron scanning microscope. It is given a separate name partly because it is used for quite different purposes and also because it is in many ways different in design and operation. It is, indeed, a 'composition-sensitive' microscope and so gives much more information about a specimen than does either the ordinary or the scanning electron microscope. A more descriptive title would be the 'electron flying-spot X-ray microanalyser', emphasising both its operation and its analysing capabilities. But this unwieldy name is usually shortened either to the 'X-ray microanalyser' or the 'electron probe microanalyser' (EPMA). The word 'probe' indicates better than 'flying-spot' its practical function, but actually derives from the fact that the first X-ray microanalyser had a stationary electron spot, the specimen being moved about with micrometers to explore different parts of it.

The original static spot microanalyser was built in France in 1949, and the first flying-spot instrument in Cambridge in 1955–56. The value of the latter is that it shows a picture of the part of the specimen actually being analysed and greatly speeds up the exploration of a sample. It has found many applications, particularly in metallurgy and mineralogy, and has established itself as an important research tool even more rapidly than did the electron microscope. A number of models are in commercial production in America, Britain, France and Japan, and they are all now of the scanning type. The complete instrument is rather elaborate, as seen in Plate 25, mainly because of the need for accurate recording equipment in addition to the basic features of an electron microscope. The latest trend is to make it still more complicated, and faster in operation, by adding a small computer which automatically processes the readings so that the output gives the exact percentage analysis of the various elements present.

How it Works

The main part of a scanning microanalyser is essentially the same as in the scanning electron microscope, as shown in Fig. 47. The evacuated column contains the electron gun,

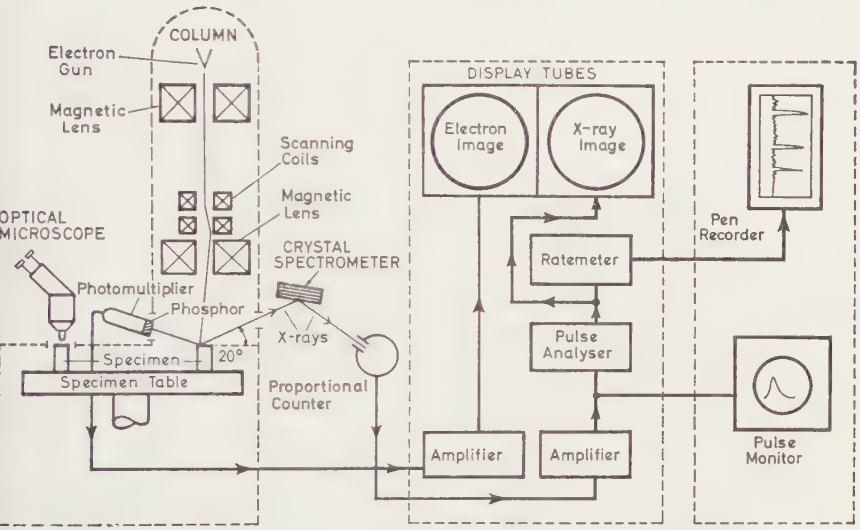

Fig. 47. Scanning electron probe microanalyser: schematic diagram.

electron lenses and deflection coils, and the specimen is inserted into the second lens. On one side of it is fitted a detector for reflected electrons, so that an 'electron image' of its surface can be displayed in exactly the same way as in the scanning electron microscope. On the other side of the column an X-ray detection system is arranged, to select particular wavelengths and to measure their relative intensities. The emitted X-rays contain the characteristic lines of all the elements present in the specimen, and also a general background of X-rays of all wavelengths, in much the same way as sunlight contains the spectrum of each element in the sun, superimposed on the white radiation. Just as a prism spectrometer is used for sorting out the visible spectrum, an X-ray spectrometer can be used to separate the different X-ray lines. Instead of a prism, the action of which depends on refraction, we use the surface of a

crystal from which X-rays are reflected in directions depending on their wavelengths, by a process of diffraction. As we saw in Chapter 3, visible light is randomly diffracted by isolated particles. When the particles are regularly arranged, as are the atoms in a crystal, diffraction takes place in particular directions which depend on the spacing of the planes of atoms as well as on the wavelength of the radiation (Fig. 48*a*).

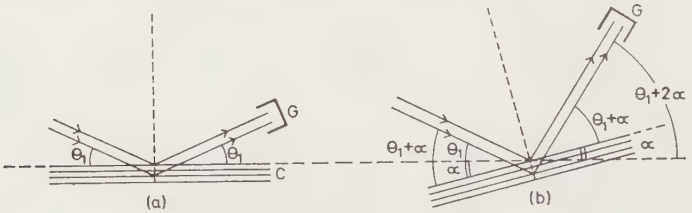

Fig. 48. X-ray crystal spectrometer principle. In (*a*) the crystal *C* is set to reflect a given X-ray wavelength into the counter *G*, at a glancing angle θ_1; in (*b*) it has been rotated through an angle α so as to reflect a longer X-ray wavelength, and *G* has to be rotated through 2α to pick up the reflected beam.

Fortunately atomic spacings are of the same sort of size as X-ray wavelengths (a few Ångström units), and the result is that diffraction takes place at reasonable angles; with visible light the angles would be impracticably large and the reflected beams would almost coincide with the incident beam.

A crystal spectrometer, then, consists of a small block of a substance that is readily ground and polished, such as lithium fluoride or quartz, mounted on an axis so that the angle it presents to the incoming X-rays can be varied. The direction of these rays is fixed by the position of the crystal with respect to the specimen. As the crystal is turned to select a new wavelength, the counter used as detector has to be moved correspondingly to intercept the reflected rays, and so must move through twice the angle the crystal moves, as shown in Fig. 48*b*. The exact wavelength of the strongest X-ray line from each element is known, and so the spectrometer can be 'tuned in' to any element we wish to detect.

Unfortunately the X-ray intensity is usually very low. For every million electrons striking the specimen only about 1,000

X-rays are produced, and these are of all wavelengths, so that very few will be of the particular wavelength we wish to detect, even when the specimen is a pure sample of an element. Still worse, the X-rays are emitted in all possible directions and our spectrometer crystal will catch only a small fraction of them. The design and location of the spectrometer is thus the key factor in an X-ray microanalyser. It is usual to have several interchangeable crystals, each specially prepared for covering a particular range of wavelengths. There may also be one counter for detecting the short wavelengths and another, with a thinner window, for the long wavelengths. The operating voltage will be set at the optimum value, calculated to produce the maximum output of the wanted X-ray wavelength relative to other wavelengths. The beam current will naturally be kept at the highest value compatible with the size of electron spot required.

But, after all these efforts have been made, the X-ray signal will still remain very weak, especially if the amount of an element present is very small. The image displayed by the X-ray channel is often almost invisible, or rather it is impossible to distinguish it against the general background of unwanted X-rays, some of which get recorded whatever precautions we take. It is for this reason that an electron collector is also provided, since the efficiency of electron reflection is enormously greater than that of X-ray production. So an electron image can always be obtained, although it does not follow that it will show where the particular element of interest is located. But it enables us to get the electron spot into best focus, as judged from the sharpness of the electron image, and also to examine the specimen for likely areas to explore with the X-ray detector. To assist this process, an ordinary optical microscope is built into the microanalyser, so as to view the specimen either during actual analysis or (as in Fig. 47) in a different position before it is rotated into the electron beam. The investigator will usually recognise from experience particular features of the specimen which serve as a guide to the location of a given element. If the wavelengths of the X-rays from these areas are then examined with the crystal spectrometer, the presence or absence of that element can be proved and its amount determined.

The X-ray image formed by a characteristic line will give direct evidence of the distribution of an element over the region of the specimen scanned. As in the electron scanning microscope the size of the area scanned can be varied at will, thus changing the magnification. Different parts of the specimen can be examined in turn, in the first place by changing the scan, and over larger distances by shifting the specimen-stage as in a light microscope.

The qualitative information about the occurrence of a given element, which is obtained in this way, may often give the research worker the answer to his immediate problem. If a more exact analysis is required, the output of the X-ray channel is switched from the display tube to a counting mechanism ('scaler') or to a pen recorder, as also indicated in Fig. 47. The electron spot is then stopped over the region of interest, perhaps a minute inclusion in the specimen, whilst the spectrometer crystal is slowly rotated through the position at which the given wavelength is detected. The operation is then repeated with a pure sample of the same element, and, by comparing the areas under the two peaks on the pen recorder trace, the amount of it present in that part of the specimen is determined. Or it may be accurate enough to leave the spectrometer at the correct setting and simply obtain two counts on the scaler, from the sample and the pure element. The process of exploration can be speeded up by scanning the electron spot very slowly along a line across the specimen, at a given spectrometer setting, whilst the pen recorder traces out the variation in X-ray output.

We have assumed so far that we are interested in a particular element and know that it is present, but there may be occasions when only the main elements present are known. The simplest way of detecting any others is then to keep the electron spot stationary and run the spectrometer through a wide range of wavelengths with the output operating the pen recorder. The presence of particular elements will then be indicated by the occurrence of peaks at the wavelengths of their characteristic lines, as in Fig. 49. The height of the peaks will give a rough estimate of their relative concentrations, but an accurate result requires the application of a number of correction factors, since

different wavelengths are emitted (and detected) with differing efficiencies and also may be absorbed to differing extents in the specimen and in the detector.

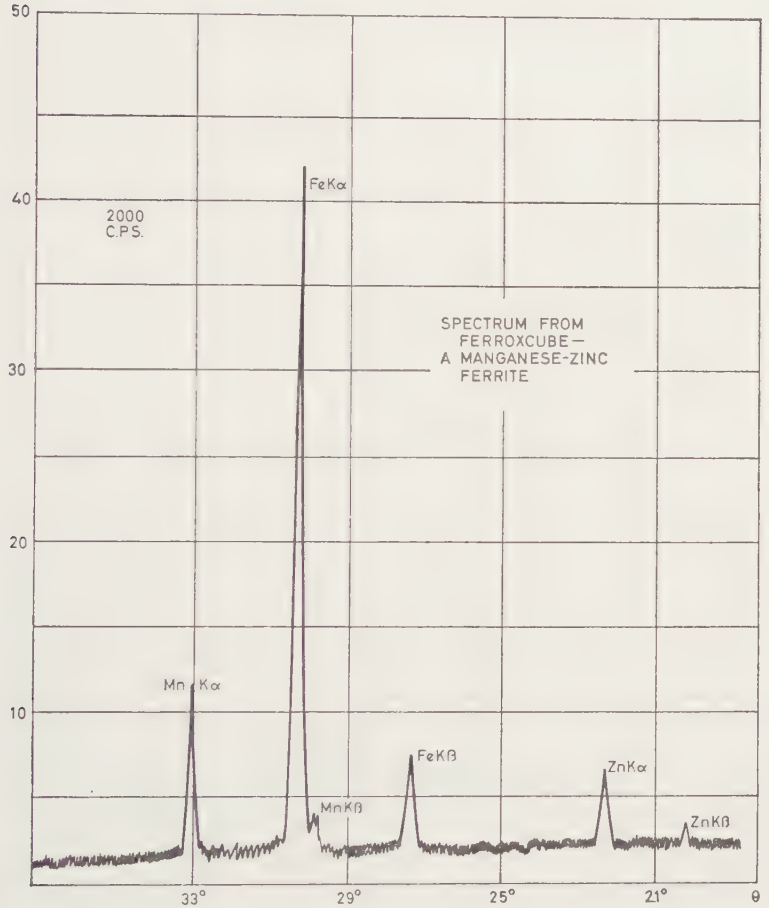

Fig. 49. Relative intensities of characteristic X-ray lines of elements present in an iron alloy, as recorded from the output of the crystal spectrometer of an electron probe microanalyser. (*From* X-ray Microscopy *by V. E. Cosslett and W. C. Nixon; University Press, Cambridge.*)

Microanalysis of Metals and Minerals

When all corrections have been made the X-ray micro-analyser gives the amount of an element present to an accuracy

of about 1 part in 1,000, and in a few specially favourable cases to a few parts in 10,000, in a spot about 1 micron in diameter and 1 micron deep. This corresponds to detecting a mass of about 10^{-15} gram in a volume of about one billionth of a cubic centimetre (10^{-12}cc.). Although there are several other methods of analysis which can detect even smaller concentrations, none of them are able to do so in this highly-localised manner. The main value of the microanalyser is for mapping the distribution of an element over a specimen on the micro-scale, and most of its many applications are based on this unique ability.

The sort of information obtained is illustrated in Plate 26, a set of images photographed from the screen of the cathode-ray tube. The specimen was an alloy of aluminium with 6 per cent of tin and the problem was to find the location of the tin. From the electron image at low magnification, we deduce that it is in the bright areas, since tin reflects electrons more strongly than does aluminium. Further investigation shows that these strips are in fact the boundaries of grains in three dimensions; in Plate 18B, a projection X-ray picture, this was more clearly visible. The X-ray image formed at the aluminium wavelength shows that this element occurs everywhere except in the boundaries, and the image at the tin wavelength confirms this conclusion. In this instance the displayed images give the required evidence and no detailed analysis need be made. The set of images on the right of Plate 26 compares the results at much higher magnification, demonstrating that the resolution of the X-ray scanning microscope is here about 1 micron and that of the electron image distinctly better than this.

Plate 27 shows the results obtained in a more complicated material. The problem here was the distribution of a variety of elements in a piece of high-temperature alloy which exhibited corrosion. The electron image shows a region of corrosion extending inward from the surface at the left. Also visible are some inclusions or precipitated particles in the main body of the metal; grain boundaries are not apparent because the specimen was not etched. The next image was obtained with a proportional counter alone, omitting the crystal spectrometer, a method used to get rough results when the X-radiation

is too weak for the spectrometer to give any image at all. In this case the counter was attached to recording gear which formed an image from the sulphur and the molybdenum X-ray wavelengths, revealing that the included particles contain one or perhaps both of these elements. The remaining images were formed by setting the spectrometer to select the titanium, chromium, cobalt and nickel X-ray lines, respectively. From these we deduce that molybdenum occurs mainly along with titanium in the metal, but appears to be absent from the surface scale. The scale itself is enriched in chromium and surrounded by material which is deficient in chromium, but contains cobalt and nickel to the same extent as in the sound metal. Grain boundaries are just visible here and there, owing to a slight enrichment in chromium and deficiency of cobalt. This sort of information was sufficient to give the research metallurgist some idea as to the processes at work during high temperature operation which were responsible for the corrosion. The amounts of alloyed elements involved were so small that it would have been impossible by any other method to detect their distribution in these minute regions.

The relative brightness of different regions of an X-ray image gives an approximate indication of the variation in concentration of the element forming it. This information can be made more quantitative if we suppress part of the image, by 'gating' the output of the amplifier so that only signals stronger than a set level are passed to the cathode-ray tube. Only areas containing more than the corresponding amount of the element will then be imaged. By double-gating, i.e. setting an upper as well as a lower limit to the signal strength, it is possible to image only those regions which contain a certain range of concentration of a particular element. Plate 28 shows an example of this technique, in which the chromium content of a steel is mapped at three levels: total, between 3 per cent and 8 per cent, and more than 10 per cent. An optical micrograph is also included, so that the distributions of chromium and phosphorus can be related to the visible surface structure of the metal. Although only the surface can be investigated in any one set of experiments, deeper parts of the metal can be

explored by etching off the surface layer with acid or by electrolysis.

By extending the method illustrated in Plate 28 to narrower intervals of signal strength it is possible to build up contour maps of the distribution of each element in the specimen. Another way of obtaining such information is shown in Plate 29. The electron probe is set to travel along a line over the specimen, selected from the electron scanning image, whilst the X-ray output signal for a given element is caused to deflect the spot of the cathode-ray tube, which is also scanning a line path. The peaks in the resulting trace, as illustrated, then give the approximate variation of the concentration of the element across the part of the specimen traversed. The amounts of several different elements can be traced in turn. In the example shown here, the question was whether the reaction product on the surface of a beryllium sample was composed of the oxide or carbide of this element. To enable a direct identification to be made, a small piece of graphite(on the right) and a still smaller piece of lithium hydroxide (to the left) were placed on the specimen, to act as reference standards for carbon and oxygen, respectively; lithium X-rays are beyond the limits of detectability. These markers show up clearly in the oxygen and carbon traces, whereas the areas covered by reaction products show mainly oxygen and only very little carbon, such as might be due to stray dust particles. Beryllium appears strongly in the corresponding trace, except where it is masked by the reference spots or the reaction products. The identity of the latter as oxide is thus convincingly proved. These results were obtained without a crystal spectrometer, using a proportional counter and special recording equipment.

So far we have considered only examples drawn from metallurgical research, involving the mapping of the distribution of elements and the identification of corrosion or other reaction products. Many other problems involving metals can be solved with the microanalyser, such as the detection of precipitate formation at a very early stage and the rate at which one metal diffuses into another during heat treatment. A variety of mineralogical problems have also been attacked, some similar to those just mentioned and others quite different.

One of the earliest successes of the microanalyser in this field was to map out the variation in relative concentrations of iron and nickel in a piece of a meteorite. Another was the location of vanadium in a sulphide ore from Finland.

In general the range of problems open to investigation in mineralogy is even wider than in metallurgy, since by the very process of their formation rocks and minerals usually contain a variety of constituents, or at the very least layers or gradations of a single substance. A striking example of this is shown in Plate 30, a set of images of the mineral bravoite which is found in certain mines in Germany. From a bulk analysis it was known to consist primarily of the sulphides of iron, nickel and cobalt, and the optical microscope showed it to have a remarkable marbled appearance, indicating that these compounds were laid down in well-defined zones. The microanalyser gave images from the nickel, iron and cobalt X-ray lines which

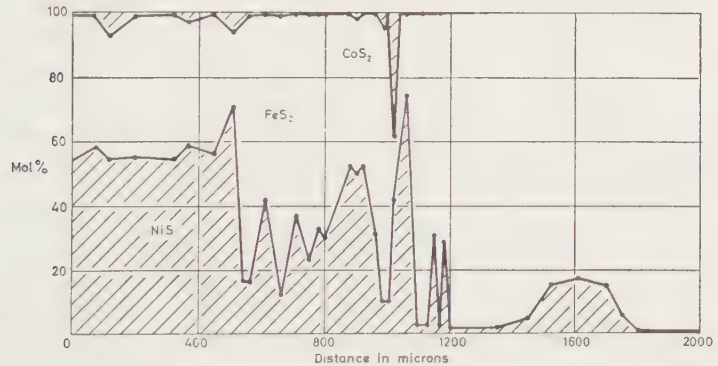

Fig. 50. Point-by-point plot of the relative concentrations of the sulphides of cobalt, iron and nickel across a mineral (bravoite) as determined with the electron probe microanalyser. (*From G. Springer and J. V. P. Long, in* X-ray Optics and X-ray Microanalysis; *Academic Press, New York and London.*)

reveal at once which elements occur in the various bands. In particular a high nickel content coincides with low reflectivity of light and a change in colouration: the colour changes from bright yellow through brown to grey with increasing nickel concentration. Spot measurements of the amount of each element were made on the various zones, allowing a concentration diagram to be drawn up (Fig. 50); since no other element

could be found with the microanalyser, the total must be 100 per cent.

The variation in composition which occurs in such a mineral as bravoite must be related to the conditions in which it was formed at some stage in the Earth's development. It is now possible to attempt to reproduce the likely conditions of temperature and pressure by means of laboratory experiments in high-pressure vessels. In the particular case of bravoite many samples were prepared with various acidities and metal-ion concentrations in the starting solutions, in the attempt to find the reasons for its zoning. The reaction temperature was also varied. The X-ray microanalyser has verified that in certain conditions it is indeed possible to synthesise this type of zoned structure in the laboratory, thus making a great advance in our knowledge of experimental mineralogy.

The example we have taken here is only one of a wide range of applications of the microanalyser in mineralogy, petrology and geology. It has become so valuable a research tool, in fact, that a special model has now been designed and produced for these applications, known as the 'Geoscan'. It allows a specimen to be inserted in the form in which minerals are normally prepared for optical examination—as a ground-down section supported in pitch or on a glass slide. The bravoite example also makes the point that the X-ray microanalyser is only one tool amongst many when it comes to solving actual research problems. One may call on chemical and other methods of bulk analysis, as well as on optical and electron microscopy. Very often an attempt to use the microanalyser alone, without the prior knowledge given by these other methods of investigation, would be rather like looking for a needle in a haystack. The great advantage of the new instrument, when properly used, is that it combines both chemical analysis and microscopy and enables us to pinpoint the occurrence of elements in a way which otherwise is impossible.

The Microanalyser in Biology

The same comments are true of its biological and medical applications, although here the range of usefulness is not yet

very well defined because they are still in an early stage of exploration. But it is obvious that the microanalyser will be useful wherever local amounts of inorganic elements occur in plant or animal tissues.

Two general types of situation can be distinguished: naturally occurring elements, such as calcium in bone and teeth, and foreign bodies, such as dust in the lungs of miners or asbestos workers. In the former situation the problem may be to determine the distribution of calcium within a given specimen (a tooth, say), and to follow the change in amount at a given site with age or the variation from individual to individual. A rather different aspect, which is at present being investigated, is the way in which calcium is deposited in the wall of the arteries in the early stages of the disease known as atherosclerosis or hardening of the arteries. The practical difficulties in such a case are much greater than with metallurgical specimens, since there is real risk that the electron probe will not only damage the sample of tissue but may even burn away parts of it before analysis can be completed. To avoid this a thin section of the artery is very lightly coated with aluminium and then mounted on a conducting base, such as a block of brass or aluminium. Protected in this way sections of soft tissues are usually quite stable and can be explored with the electron probe for long periods, partly because most of the current goes right through the section into the supporting base.

The study of hard tissues, such as tooth and bone, gives rise to further difficulties. Not only is it less easy to make thin sections, but these are much less readily penetrable by the electron beam. So less use has so far been made of the microanalyser in this field, although a few interesting results have been forthcoming. One problem solved, for instance, was the cause of the red colouration long observed in the teeth of rats and a number of other rodents. It had been suggested that it might be due to an iron compound, and the microanalyser was able to show that this is indeed so. The image obtained from the iron X-ray line (Plate 31) demonstrated clearly the presence of iron in the enamel of a typical tooth, and the recorder trace confirmed that it occurs in a sharply defined layer. From the

height of the peak it is calculated that the amount of iron is as high as 7 per cent. The calcium X-ray image does not show a corresponding depletion in this layer, mainly because the enamel is slightly porous and so does not give a uniform distribution of the element. There is, however, a drop in calcium content between the enamel and the underlying dentine, which shows up more clearly in the pen trace than in the calcium X-ray image.

The use of the microanalyser for identifying foreign bodies is more straightforward. It is only necessary to cut a section from the suspected tissue, lightly metallise it and explore it with the electron scan whilst the spectrometer is set for the suspected element. One of the very first uses of the scanning microanalyser was in fact to locate tin oxide particles in the lungs of a patient who had died with unusual symptoms. In the same way one can determine the amount of an element which occurs naturally in the body, such as iron in the liver or in blood cells, or an unnatural amount such as the deposition of copper in a condition of the eye known as Wilson's disease. There is indeed considerable scope here for the microanalyser to map out the occurrence and distribution of metallic compounds in the normal tissues and organs, so that abnormal conditions can the more readily be recognised. It is known, for instance, that zinc occurs in measurable amount in the skin but there seems to be little knowledge about its distribution or possible variation in different types of skin disease.

The location and analysis of organic compounds, which contain little or no metallic elements, is a much more difficult matter. Analysis of the elements which mainly compose plant and animal tissues—carbon, nitrogen, oxygen—is in any event not easy because they emit X-rays very weakly and at very long wavelengths which are difficult to detect. More fundamental is the fact that several different compounds are likely to occur in the same part of a tissue, and they differ mainly in having the same elements differently arranged. Special attachments for the microanalyser have been devised for analysing carbon, nitrogen and oxygen in inorganic materials, and even beryllium, as we saw in connection with Plate 29, but there is very little immediate hope of being able to

distinguish one organic compound from another by direct attack in the microanalyser.

An indirect method, however, can sometimes be more successful and a useful line is suggested by the staining procedures developed long since for recognising substances in the light microscope. It was found that particular constituents of a cell would absorb or react with a given chemical dye, and in this way we can make visible their distribution by staining sections appropriately. The same principle is applied in electron microscopy, by treating the specimen with compounds of heavy elements which produce high contrast in the image. In the X-ray analyser we need only find some means of attaching almost any metallic compound to one part of a cell (for instance) rather than another. On the basis of experience of staining for the light microscope, a great number of such preferential stains are known and it is simply a matter now of adapting them to the special conditions of preparing specimens for the microanalyser.

An example of this, one of the first attempted, is the localisation of a certain enzyme connected with the process of energy liberation in the cell, and known as alkaline phosphatase. The staining procedure for detecting it results in the formation of cobalt sulphide, which is black and so is visible in the light microscope if sufficient is deposited by the reaction. With the scanning microanalyser there is no possibility of confusing this with any other substance, since we can form images with either the cobalt or the sulphur X-ray line. The regions which contained the enzyme are thus revealed very clearly, as shown in Plate 32 (*A* and *B*). A more quantitative measure of its distribution is given by a line scan across the centre of the cell, as the traces in (*C*) and (*D*) show. This experiment was made on a section of rat kidney, but the same method can equally be applied to any other tissue in which the enzyme occurs. It seems probable that the X-ray microanalyser will be able to detect much smaller quantities than is possible by optical observation.

These few examples show something of the range of uses of the microanalyser in biological and medical research, but the possibilities are only just beginning to be explored. If the

methods of specimen preparation can be perfected, if we can avoid all danger of damage in the electron beam and especially if the staining procedure can be extended to other constituents of the cell, its scope will become comparable with that of the electron microscope. Because of its ability to give chemical information about microscopical structures, its ultimate contribution to research may be even greater in the long run. But that eventuality will depend on how far the resolving power, and with it the detection sensitivity, of the microanalyser can be further improved.

The Future of the X-ray Microanalyser

As an X-ray microscope, the scanning microanalyser is limited by the electron intensity which can be focused into a sufficiently small spot, in much the same way as is the scanning electron microscope. But the limitation sets in sooner for X-ray imaging because the efficiency of characteristic line production is so much less than that of electron reflection, and so the X-ray signal for a given beam current is much weaker than is the electron signal, as we saw earlier. The first step towards improving the performance of the X-ray scanning microscope must thus be to improve the collection and selection of the X-rays. At the moment our spectrometers are able to pick up only a very small fraction of the X-radiation coming out of the electron spot, usually less than one-thousandth. There is great scope here for redesigning the spectrometer-counter system. A much larger crystal could be used, for instance, but then it has to be bent into the shape of a concave mirror so as to reflect the X-rays uniformly into the counter, and this is technically difficult to achieve. It would be even better to have some new type of counter, perhaps a solid-state device, which would select X-ray wavelengths as well as detect them, so that we could dispense with the crystal spectrometer altogether. Some progress is being made in this direction, but the counters so far produced do not discriminate finely enough between one X-ray line and the next.

As a microanalyser, the X-ray scanning system is not quite so severely limited as it is for image formation. The main

factors now are on the one hand the length of time we are willing to wait for an analysis, and on the other hand the minimum X-ray signal which can be detected against the general background of the counter output. In general, the longer count we take at a given wavelength, with the spot stationary on a particular point of the specimen, the more accurate is the analysis. But a time of 10 minutes per point is already long, since it is usually necessary to explore many points, and 30 minutes would be tolerable only where the result is of the greatest importance. For observations over such a period, the stability of the electronics and especially of the amplifier becomes something of a problem. In any event there is always a general background in the counter output, due to the 'white' X-radiation previously mentioned, a slight overlap from neighbouring X-ray lines, and the cosmic rays. In pushing an analysis to the limits, therefore, we have to keep an eye on all these factors in order to avoid putting too much effort into suppressing one when an irreducible limit is being set by another.

In face of these difficulties it is a tough battle to improve greatly on the present performance of the scanning microanalyser. One or two experimental instruments have achieved a resolving power of 0·1 micron, thus reaching the same limit as the ultra-violet and the X-ray projection microscopes. This is done on a specimen consisting of small particles supported on a plastic film, so that there is negligible X-ray background from around the particles. In these conditions less than 10^{-15} gram of an element can be detected. To progress much farther we have to hope for improvements along the lines mentioned in connection with the electron scanning microscope: electron guns of greater intensity and electron lenses corrected for spherical aberration. We might then expect the X-ray scanning microanalyser to get down to a resolving power of 200–300 Ångström units, in due course, but the ultimate attainment of 100 Å, the present limit for the electron scanning microscope, remains very debatable.

In the meanwhile much attention is being given to combining the microanalyser with the electron microscope. As we noted early in this Chapter, the electron gun and lenses of the former

are similar to those in the latter, the main difference being that the first condenser is weak and the second strong instead of vice versa. So an electron microscope can be converted for X-ray microanalysis by some slight changes to its lenses and by providing an exit window for X-rays from the specimen, together with a spectrometer and counter for analysing them. This has been done with one or two electron microscopes recently, but the arrangement is rather unhandy. It is better to start afresh and design a combination of the two instruments from scratch. One of these new hybrids is EMMA, the 'electron-microscope-microanalyser', which has been built at the Tube Investments Research Laboratory, near Cambridge. Its performance is being watched with interest. Clearly it would be of great value if we were able to identify the chemical elements in any feature of the specimens which we examine routinely in the electron microscope—for instance, in the precipitates and grain boundaries in the micrographs shown in Plates 11A and 11B. This would remedy one of the major deficiencies of the electron microscope, that it shows the form but not the chemical nature of different parts of a specimen.

EPILOGUE

Along with the new types of microscope described earlier in this book, and other research instruments such as the mass spectrometer and the electron spin resonance apparatus, the X-ray microanalyser is enabling us to reveal ever finer details of the micro-architecture of both living and inorganic Nature. It is now about 350 years since the compound light microscope was invented, and exactly 300 years since Hooke published the first systematic studies made with it. We are not yet near the end of the way which he and Leeuwenhoek pioneered, and it would be rash to attempt to predict what new discoveries are in store for us, even in the next thirty years, bearing in mind that this is only the present age of the electron microscope. The major gap which remains to be filled is that between the atom and the molecule. In special types of specimen we can detect individual atoms with the point emission microscope, and planes of atoms with the electron microscope. The latter can also show us the main features of some macromolecules. The next task must be to refine our techniques of preparation, and improve our microscopes, until we can detect the arrangement of the individual atoms in a molecule. This goal lies right at the limits of what even the optimists in our research laboratories would regard as achievable. Let us hope that they, and not the pessimists, will be proved right and that the goal will be reached within the lifetime of the reader, if not in that of the author himself.

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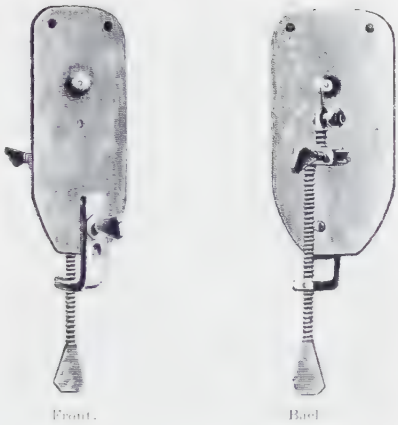
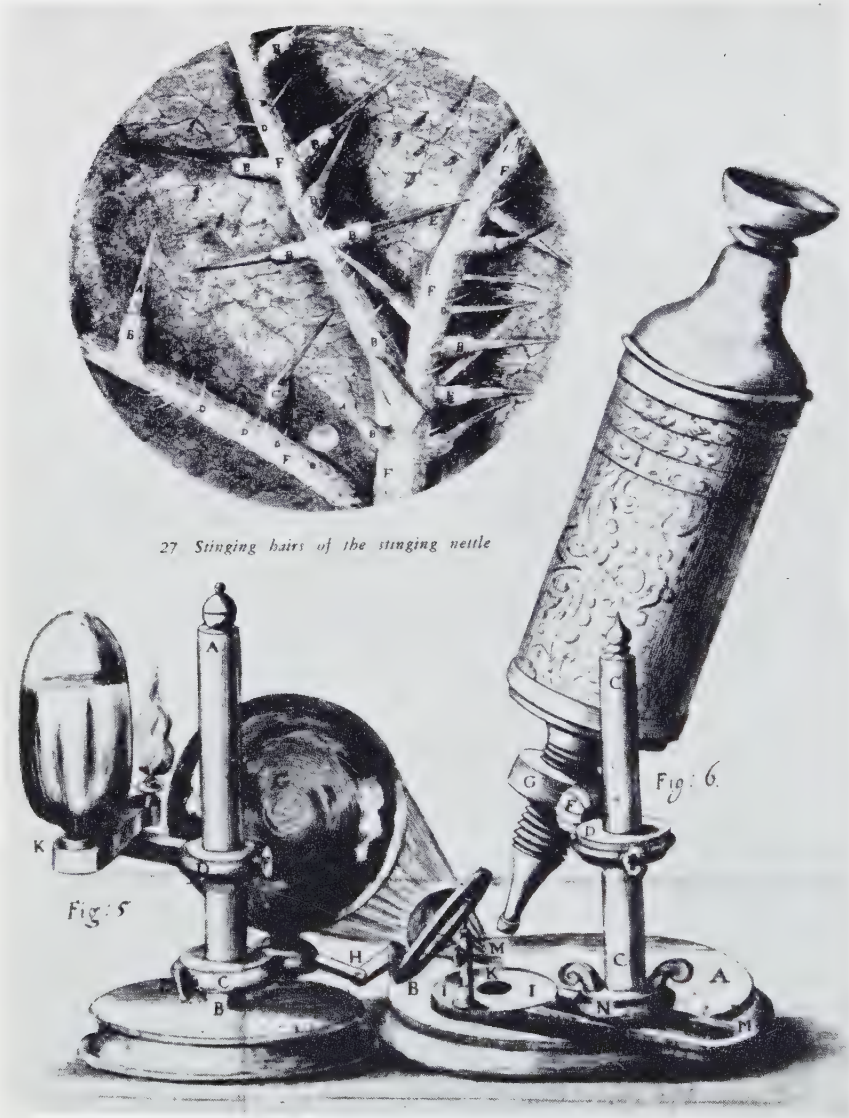
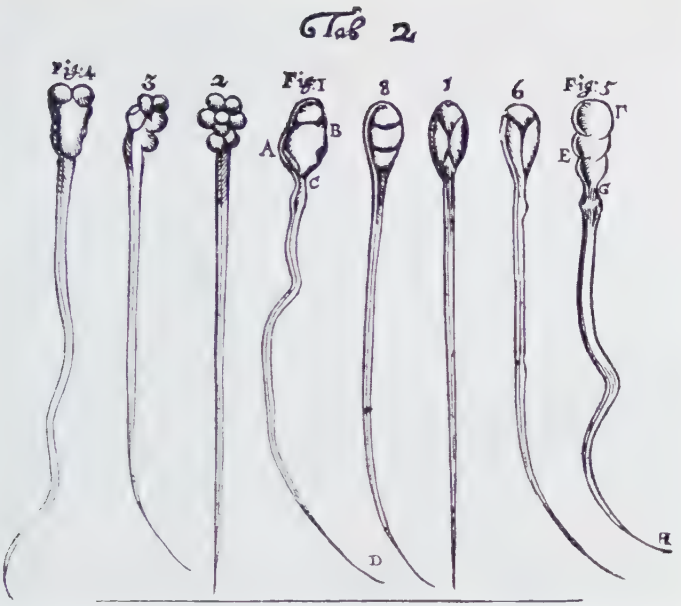


1A. Leeuwenhoek's simple microscope (half actual size). The specimen was placed on the needle point in front of the glass bead which served as lens. (*Royal Microscopical Society*)

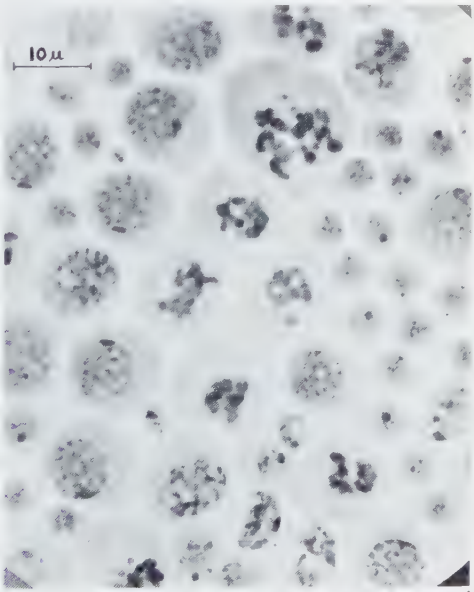
1B. A typical modern light microscope



2. Hooke's compound microscope: the specimen, mounted in a holder, M, was illuminated by the light from a lamp, K, focused by the waterfilled globe, G.
Upper left: Hooke's drawing of stinging-nettle hairs.

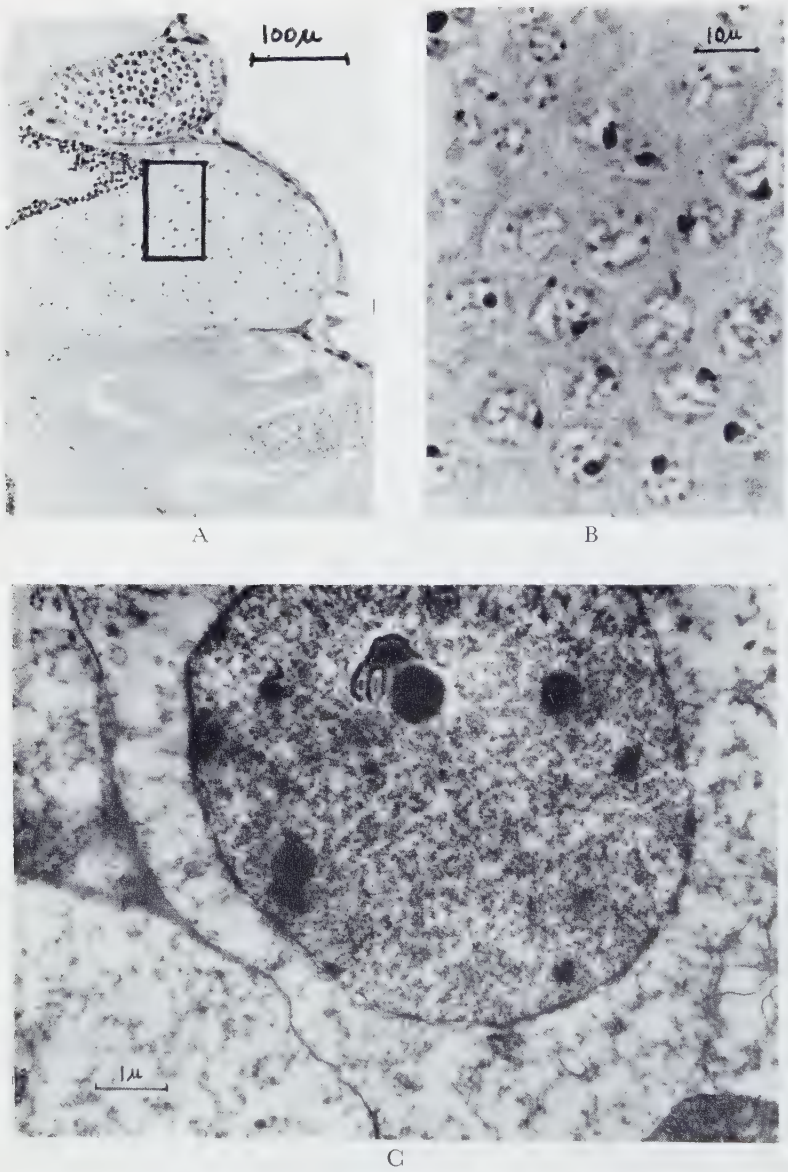


3A. Spermatozoa, as drawn by Leeuwenhoek in 1679: figures 1–4 are human, and 5–8 of the dog (approximately × 1,300).



3B (on left). Spermatozoa of the rat, as drawn by Retzius in 1909 (approximately × 1,000).

3C. Ultra-violet microscope image of locust sperm cells, showing chromosomes; compare with Plates 4 (A, B) and 6 (C, D) (× 1,000). (*Dr. Anna Cosslett, Cavendish Laboratory, Cambridge*)

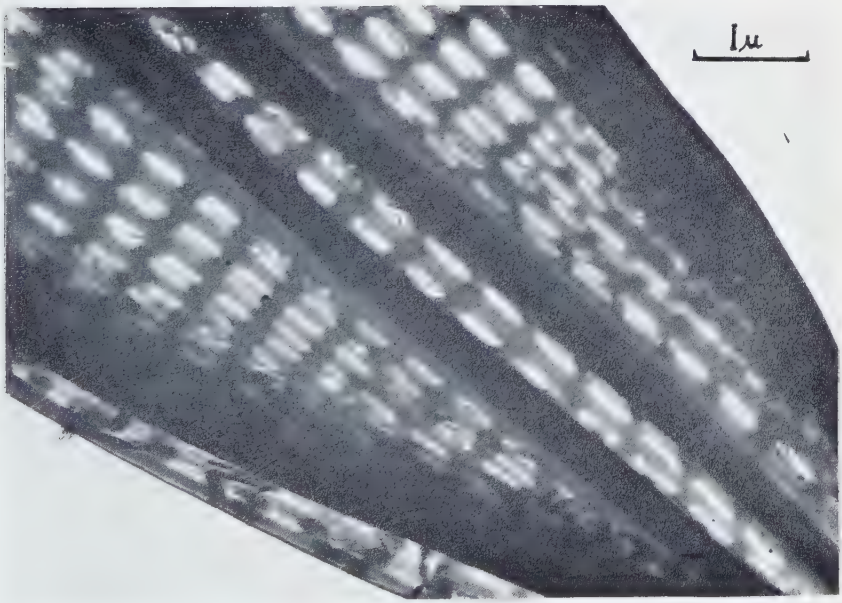


A B C

4. Sections through sperm cells of the cricket, stained to show the chromosomes, at different magnifications:
A: In the light microscope at low power (×120).
B: In the light microscope at high power; field outlined in A (×750).
C: In the electron microscope; a very thin section, showing structure in the sex chromosome (top centre) (×9,400).
(*Mrs. F. Hawkes, Cavendish Laboratory, Cambridge*)

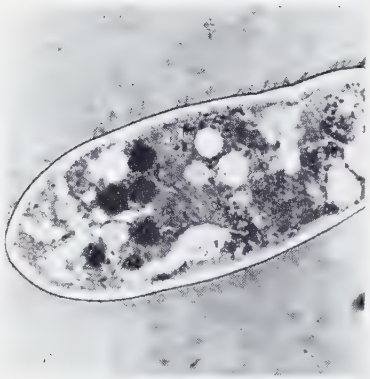
A

B

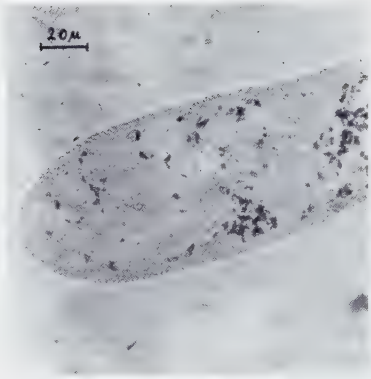


C

5. Fresh-water diatoms from Nine Wells, Cambridge, imaged in different ways, with increasing resolving power:
A: In a light microscope with numerical aperture 0·5 (× 1,300)
B: In a light microscope with numerical aperture 1·3 (× 1,300)
C: In an electron microscope (× 15,000)
(Dr. Anna Cosslett and Mr. J. Bell, Cavendish Laboratory, Cambridge)

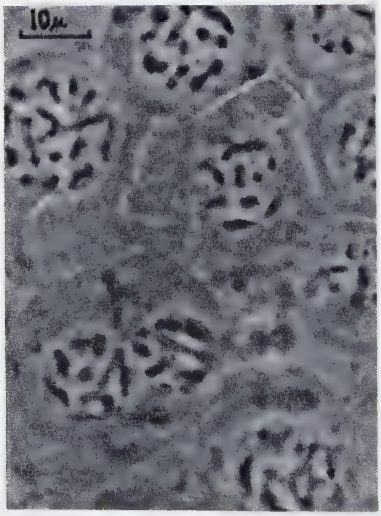


A

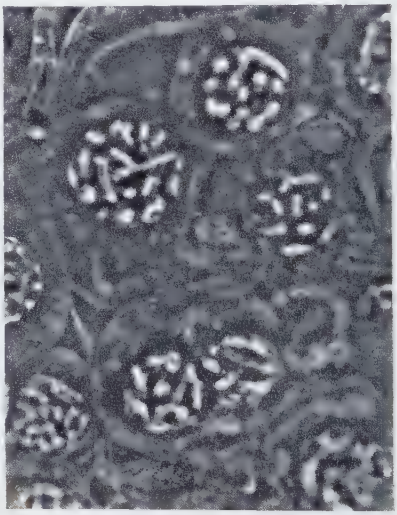


B

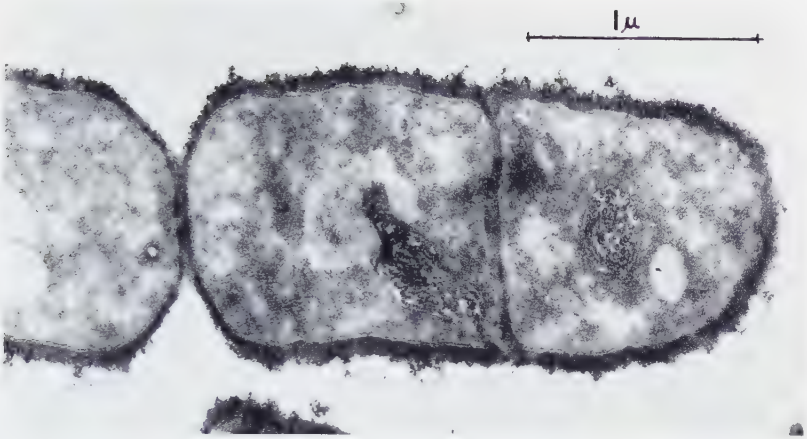
6A and B. Interference microscope image, A, and normal light microscope image, B, of the slipper protozoon, *Paramecium Aurelia* (×280).



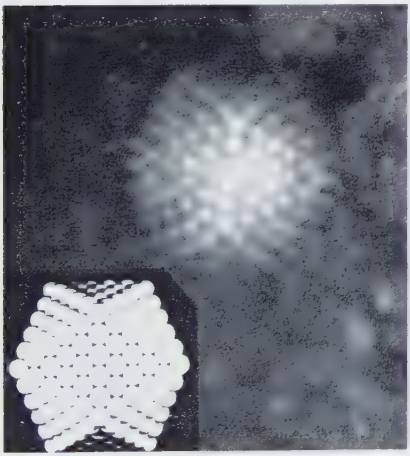
C



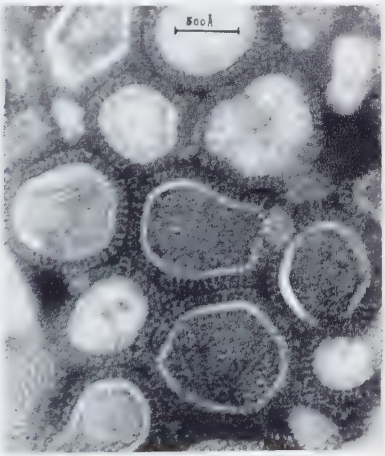
6C and D. Phase Contrast microscope images of locust sperm cells, showing chromosomes.
C: Positive contrast (×870). D: Negative contrast (×870).
(*Dr. Anna Cosslett, Cavendish Laboratory, Cambridge*)



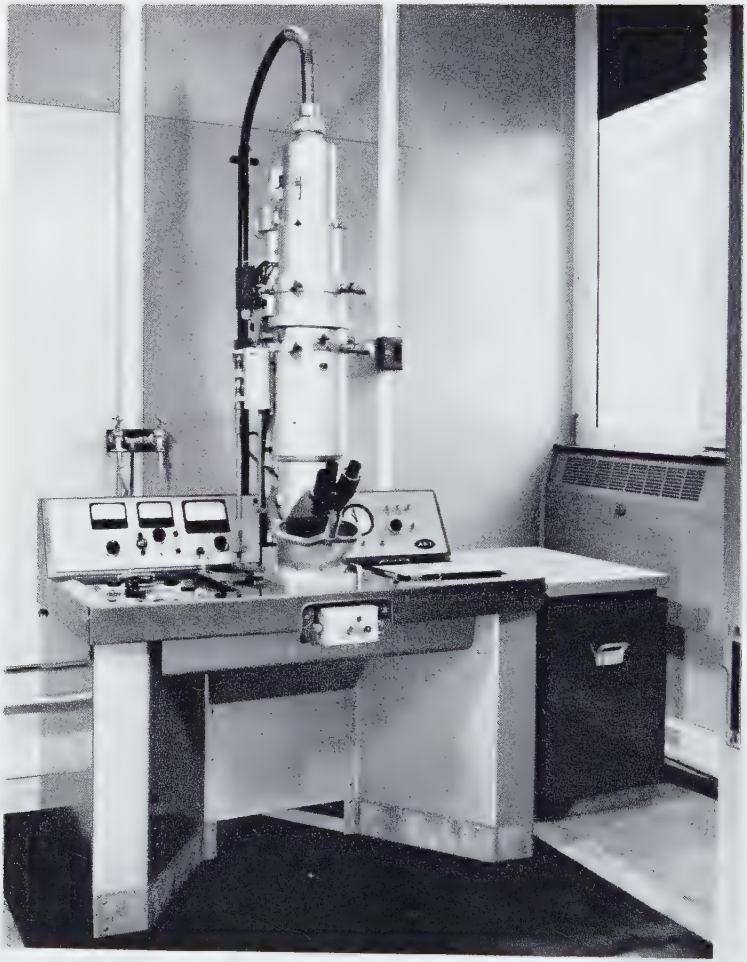
7A. Electron micrograph of section of newly divided cells of *Bacillus megaterium* after 'positive' staining (×30,000). *Miss Audrey M. Glauert, Dr. K. McQuillen and Mr. R. A. Parker, Strangeways Laboratory, Cambridge*)



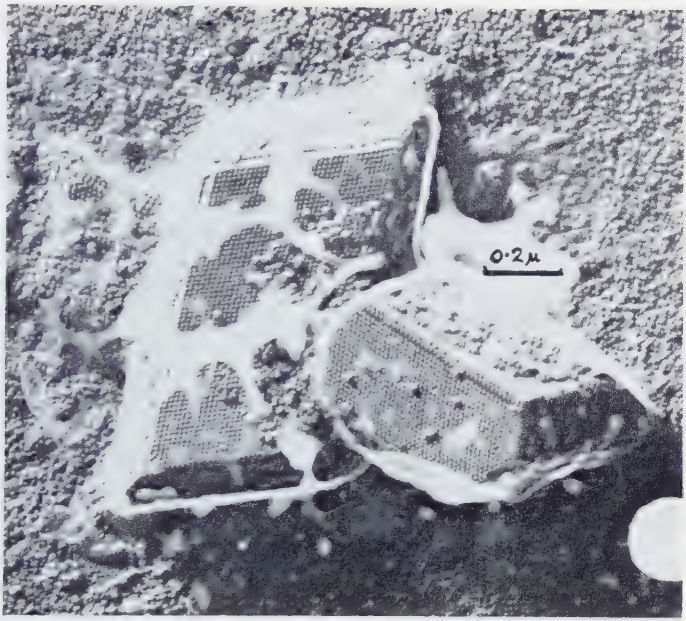
7B. Single particle of adeno-virus (about 700 Å across), as seen in the electron microscope (×400,000), compared with a ball-model of an icosahedron. (*Mr. R. W. Horne, Dr. S. Brenner, Dr. A. P. Waterson and Dr. P. Wildy, Cavendish Laboratory and Department of Pathology, Cambridge University*)



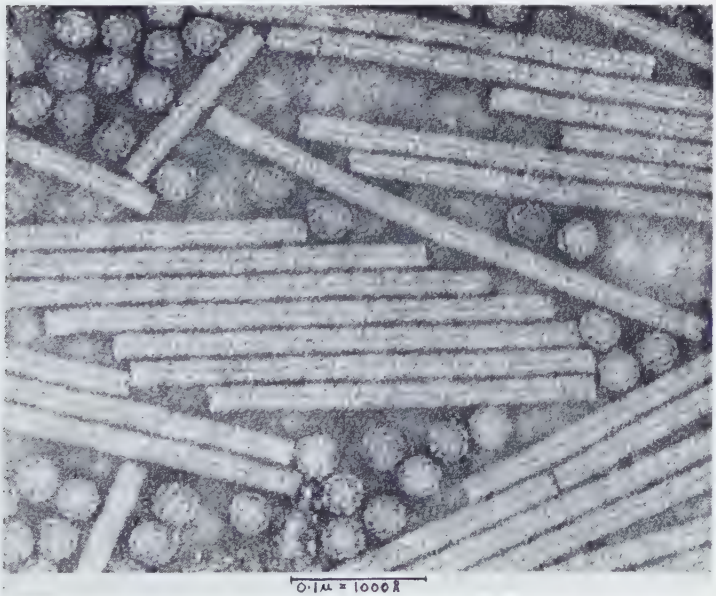
7C. Influenza virus particles imaged with the electron microscope (· 170,000). (*Mr. R. W. Horne, Dr. A. P. Waterson, Dr. P. Wildy and Dr. A. E. Farnham, Cavendish Laboratory and Department of Pathology, Cambridge University*)



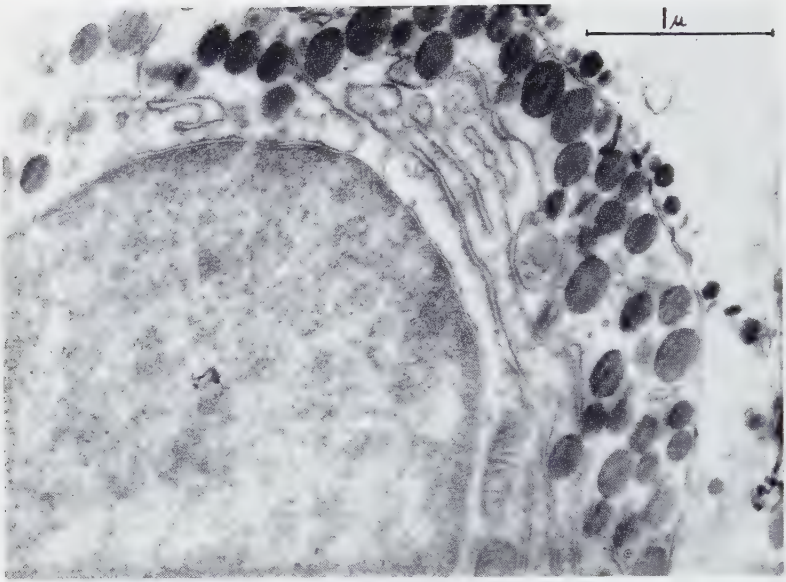
8. A modern electron microscope. (*Associated Electrical Industries Ltd., Model EM6B*)



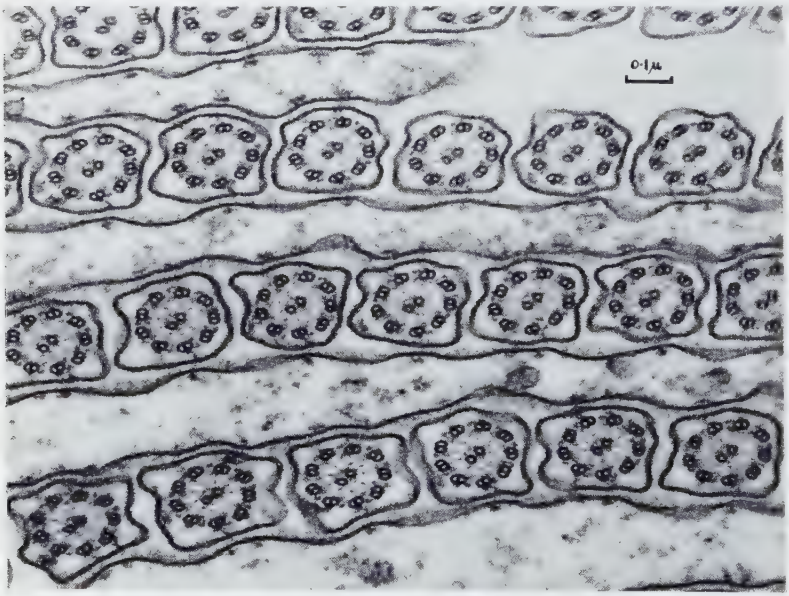
9A. Crystals of tobacco necrosis virus imaged by means of a carbon replica in the electron microscope (× 50,000). (*Dr. L. W. Labaw, Dr. R. W. G. Wyckoff, National Institutes of Health, Bethesda, Maryland*)



9B. Electron micrograph of tobacco mosaic virus (hollow rods) and turnip yellow mosaic virus (spheres), after 'negative' staining (× 175,000). (*Mr. R. W. Horne, Cavendish Laboratory, Cambridge University*)

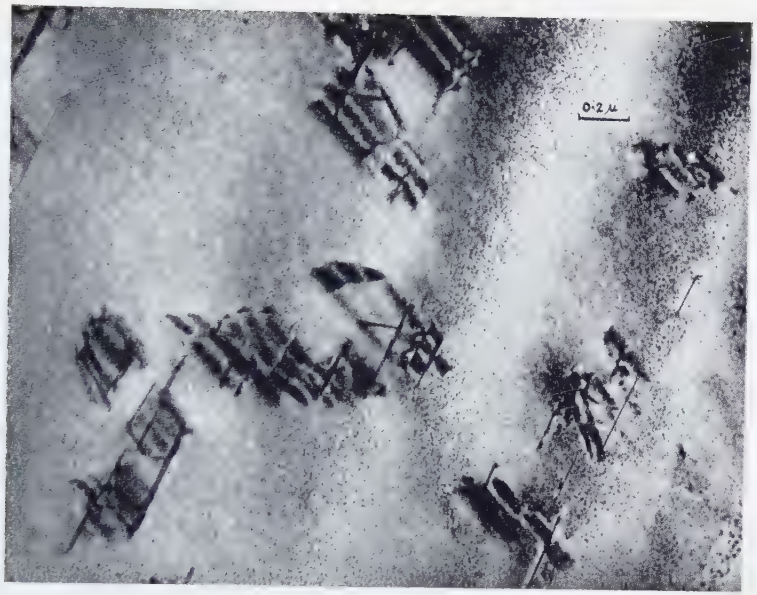


10A. Section (one of an ultra-thin series) of pituitary gland of the mouse (×24,000); the large, almost featureless area is a cell nucleus. (*Dr. Barbara G. Barnes, Cavendish Laboratory, Cambridge University*)

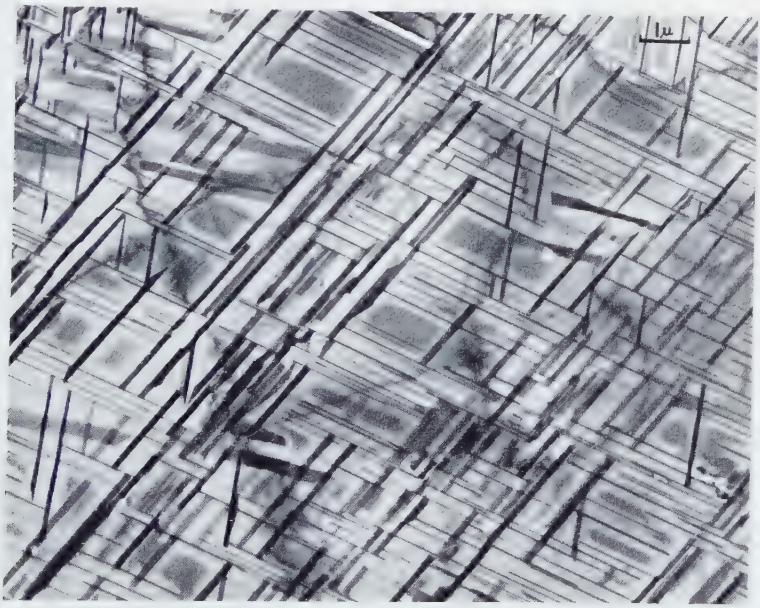


10B. Cross-section of flagella ('tails') of a protozoon (*Trichonympha*), showing central filaments surrounded by nine doublet filaments (×60,000). (*Dr. A. V. Grimstone, Department of Zoology, Cambridge University*)

A

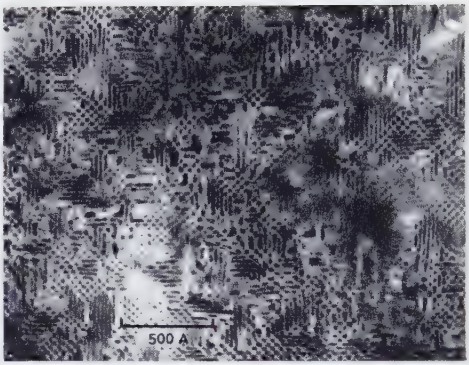


B

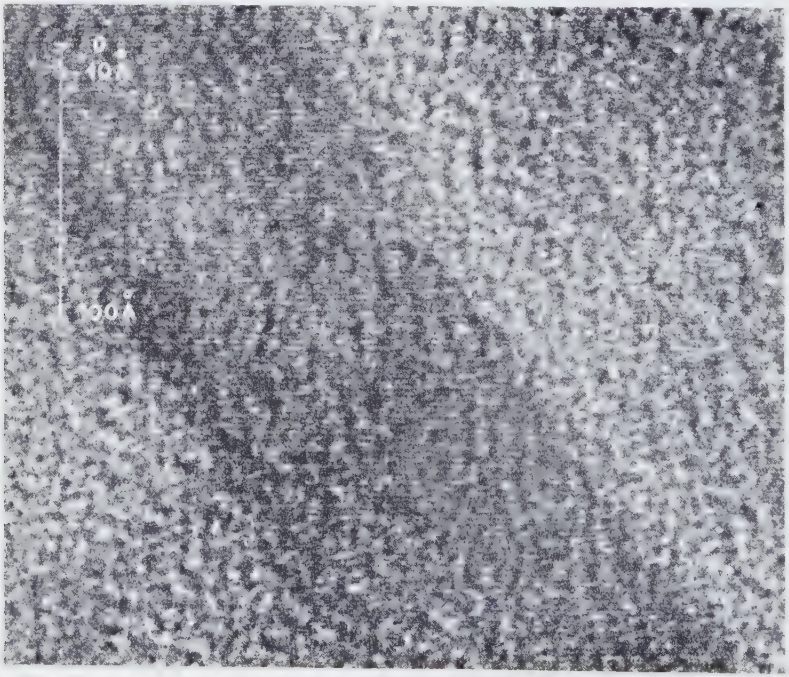


11. Formation of precipitates in an aluminium-16 per cent silver alloy, after ageing at 160°C. for A: 4 days, and B: 100 days; electron micrographs of thin foils (A: × 32,000, and B: × 6,400). (*Dr. R. B. Nicholson, Department of Metallurgy, Cambridge University*)

12A. Dislocations in thin film of stainless steel, as seen in the electron microscope (×26,000). A and B are extended dislocations (stacking faults); C is a grain boundary; other dislocations occur, individually or in rows. (*Dr. P. B. Hirsch, Mr. R. W. Horne and Dr. M. J. Whelan, Cavendish Laboratory, Cambridge University*)

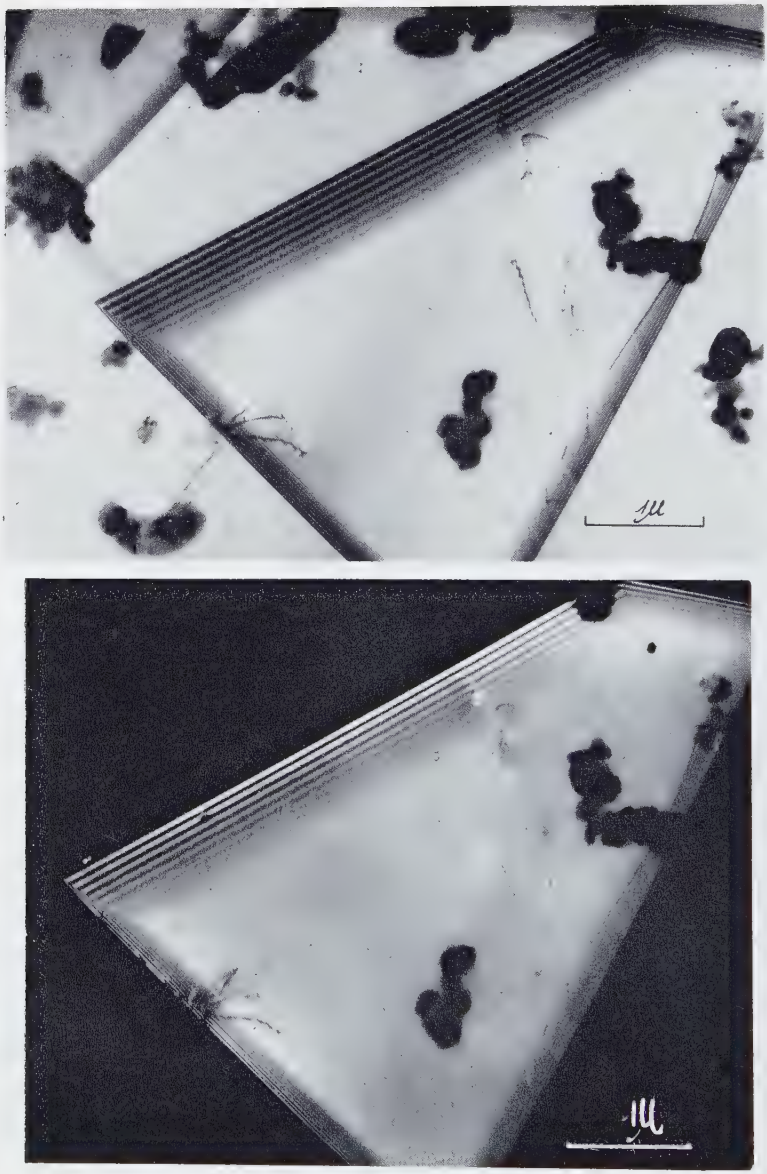


12B. Superlattice pattern formed during the transformation of a copper-gold alloy from one type of structure to another by heat treatment (×240,000). (*Dr. D. W. Pashley and Dr. A. E. B. Presland, Tube Investments Research Laboratories, Hinxton, Nr. Cambridge*)

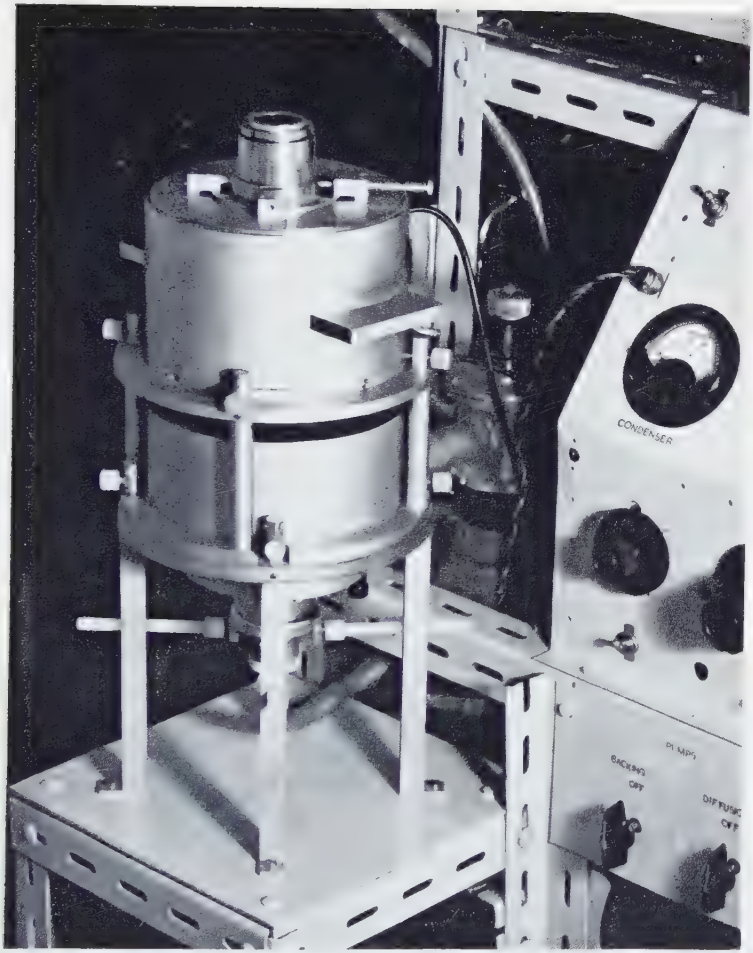


12C. Electron micrograph of a very thin gold film, showing the best resolving power so far achieved: the fine lines are planes in the gold crystalline lattice with a separation of 2·04 Å (×3,500,000). (*Dr. T. Komoda, Hitachi Company Laboratories, Tokyo*)

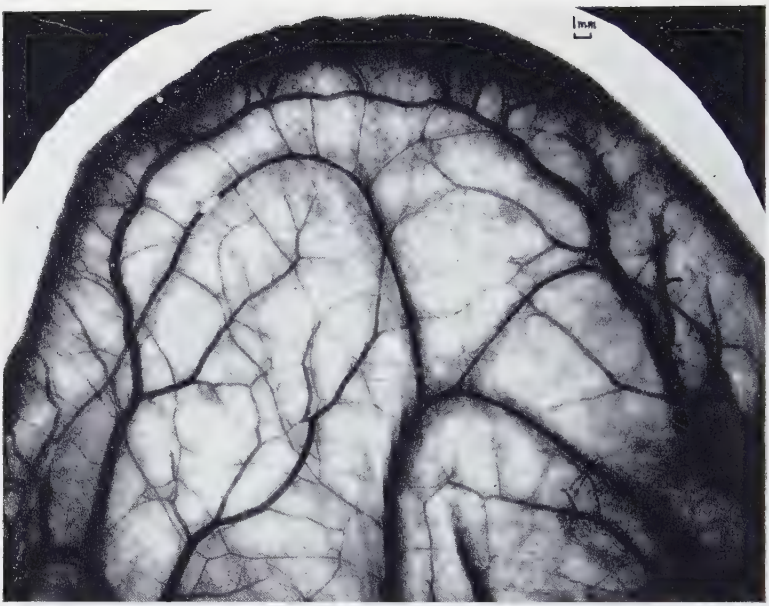
13. The French high voltage electron microscope, which has operated up to 1·2 million volts. (*Professor G. Dupouy, Electron Optics Laboratory, Toulouse*)



14. Thin foil of stainless steel, imaged in the Toulouse high voltage electron microscope at 750 kV.
A: Normal image. B: Dark-field image. (× 16,000)
(*Professor G. Dupouy, Electron Optics Laboratory, Toulouse*)

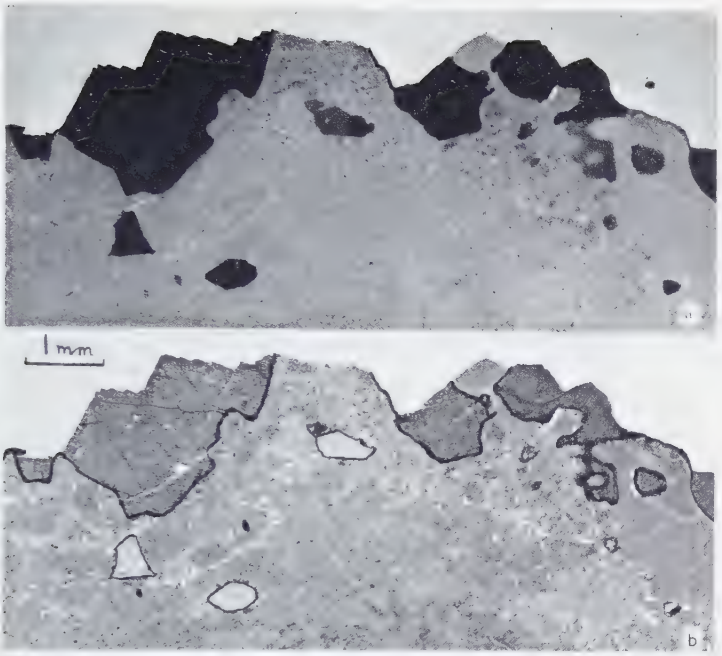


15. Projection X-ray microscope. The electron gun is below the two cylindrical electron lenses; the magnifying glass at the top is used for observing the X-ray image on a fluorescent screen. Compare with text-figure 38. (*Dr. W. C. Nixon, Cavendish Laboratory, Cambridge University*)



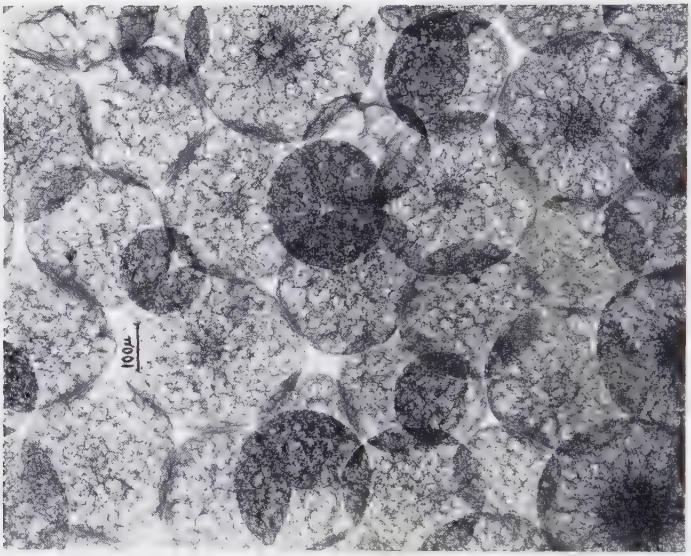
16A. Rabbit's ear, imaged with the projection X-ray microscope, showing fine details of the blood circulation ($\times 2$). (*Professor R. L. de C. H. Saunders, Department of Anatomy, Dalhousie University, Canada*)

16B. Fruit-fly (*Drosophila melanogaster*) imaged with the projection X-ray microscope showing (at right) the multiple nature of the eyes ($\times 60$). (*Dr. W. C. Nixon, Cavendish Laboratory, Cambridge University*)

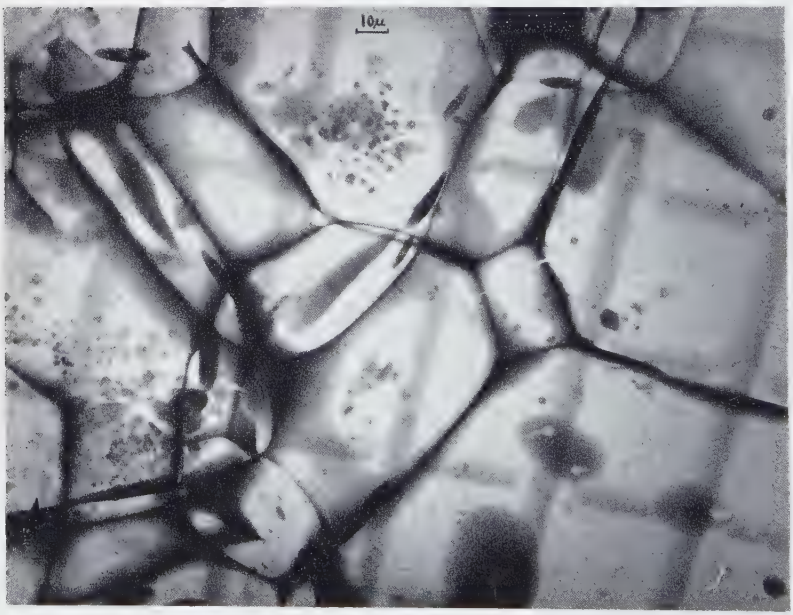


17A. A titanium-bearing ore, imaged in the X-ray projection microscope with two different X-ray wavelengths (× 10).
a: In copper radiation. b: In titanium radiation.
The titanium rich regions show up transparent in b. (*Dr. V. M. Mosley and Dr. R. W. G. Wyckoff, National Institutes of Health, Bethesda, Maryland*)

17B. Spider's knee, imaged with the projection X-ray microscope (× 25). (*Dr. W. C. Nixon, Cavendish Laboratory, Cambridge University*)

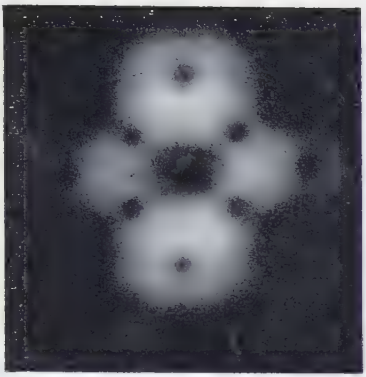


18A. Beads of polystyrene, after expansion by heat treatment, as seen in the X-ray projection microscope ($\times 60$). (*Dr. S. B. Newman, National Bureau of Standards, Washington*)

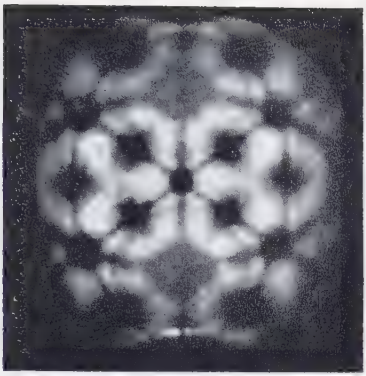


18B. Grain boundaries in a thin foil of an aluminium-tin alloy, as shown by the X-ray projection microscope: the greyish straight lines are due to the supporting grid ($\times 400$). (*Dr. W. C. Nixon Cavendish Laboratory, Cambridge University*)

A

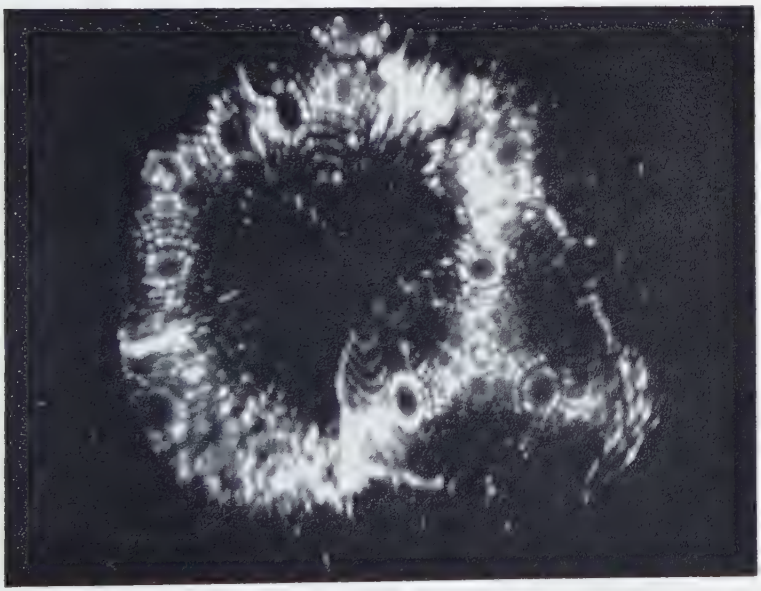


B



19A and B. Pattern formed in the field emission microscope ($\times$ 100,000). A: By a tip of pure tungsten. B: By a tungsten tip after barium has been deposited on it.
(*Professor E. W. Müller, Pennsylvania State University*)

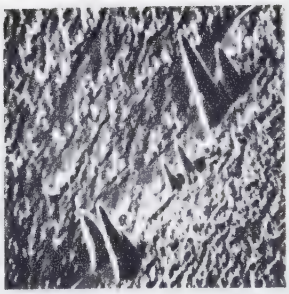
C



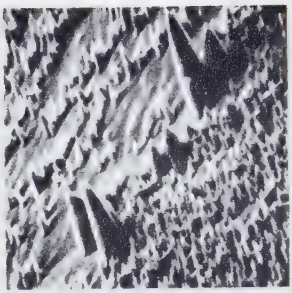
19C. Surface crater produced in a tungsten tip by the impact of a fission fragment from uranium ($\times$ 2,000,000 approx.) (compare with Plate 20). (*Dr. K. M. Bowkett, Dr. L. T. Chadderton, Dr. H. Norden and Dr. B. Ralph, Cavendish Laboratory and Department of Metallurgy, Cambridge University*)

20. Image of a tip of pure tungsten, formed in the field ion microscope at the temperature of liquid neon by means of helium ions. The smallest bright spots correspond to individual tungsten atoms ($\times$2,500,000 approx.). (*Dr. K. M. Bowkett and Dr. B. Ralph, Department of Metallurgy, Cambridge University*)

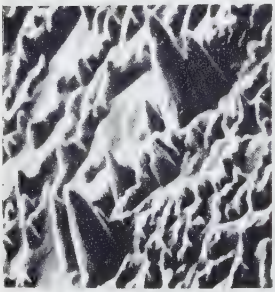
21. Flying-spot light microscope (*Rank-Cintel Ltd.*)



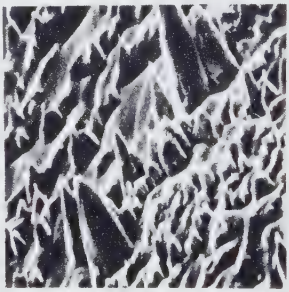
X 1380 5·4 μ t = 1540 sec.



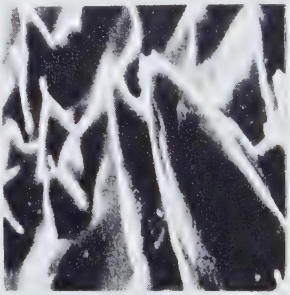
X 1380 5·4 μ t = 2000 sec.



X 1380 5·4 μ t = 2185 secs.

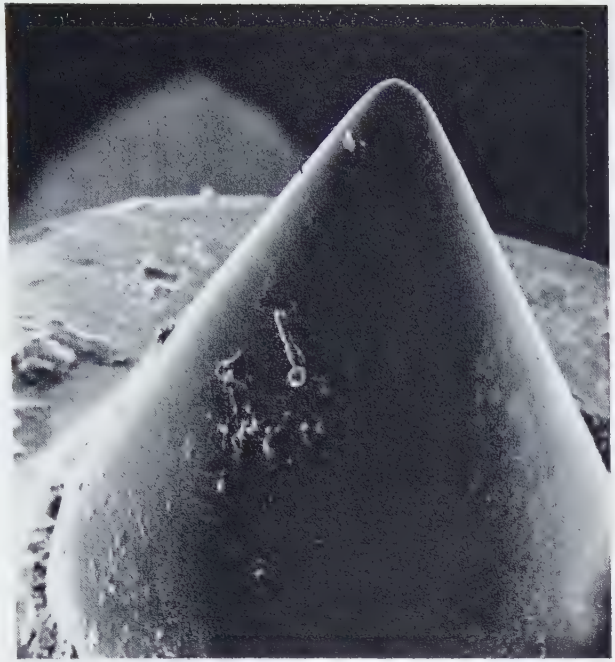


X 1380 5·4 μ t = 2620 sec.



X 3450 2·2 μ t = 2620 sec

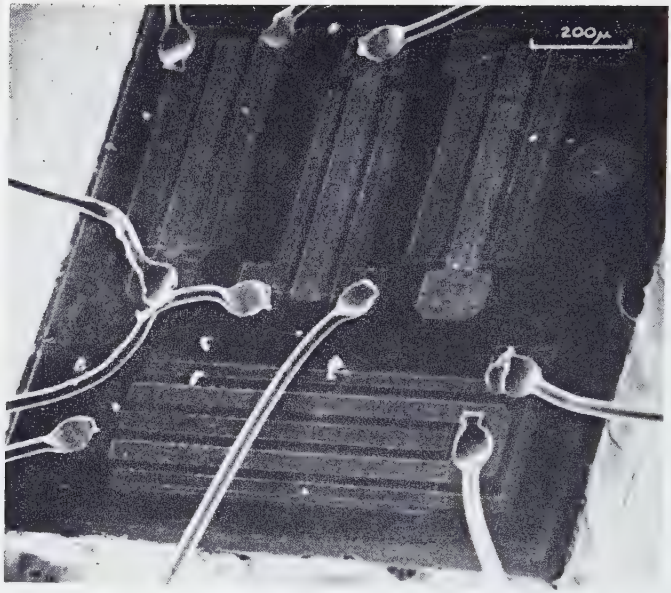
22. Scanning electron microscope images showing the progressive etching of the surface of cast tin by bombardment with argon ions (magnifications as indicated). (*Dr. A. D. G. Stewart, Engineering Laboratory, Cambridge University*)



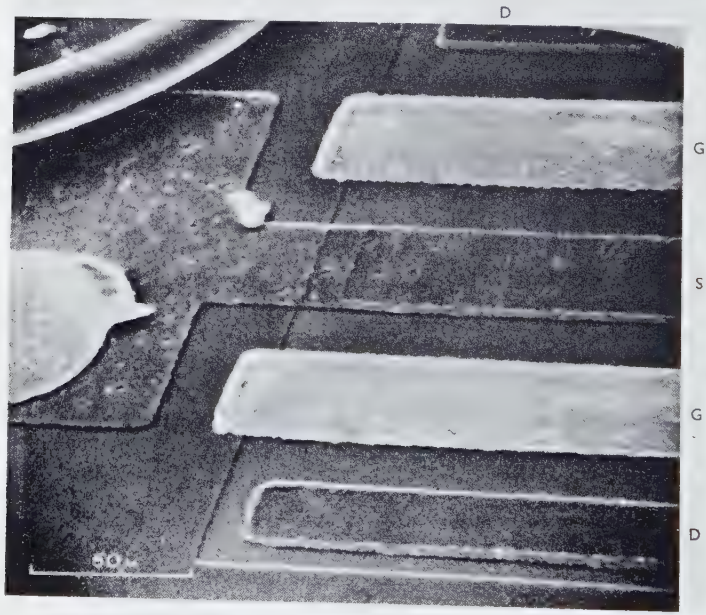
23A. Sapphire gramophone needle, imaged in the scanning electron microscope (× 150). (*Dr. K. C. A. Smith, Pulp and Paper Research Institute of Canada, Montreal*)

23B. Surface of spruce wood, after splitting, imaged in the scanning electron microscope (× 1,500). (*Dr. K. C. A. Smith, Pulp and Paper Research Institute of Canada, Montreal*)

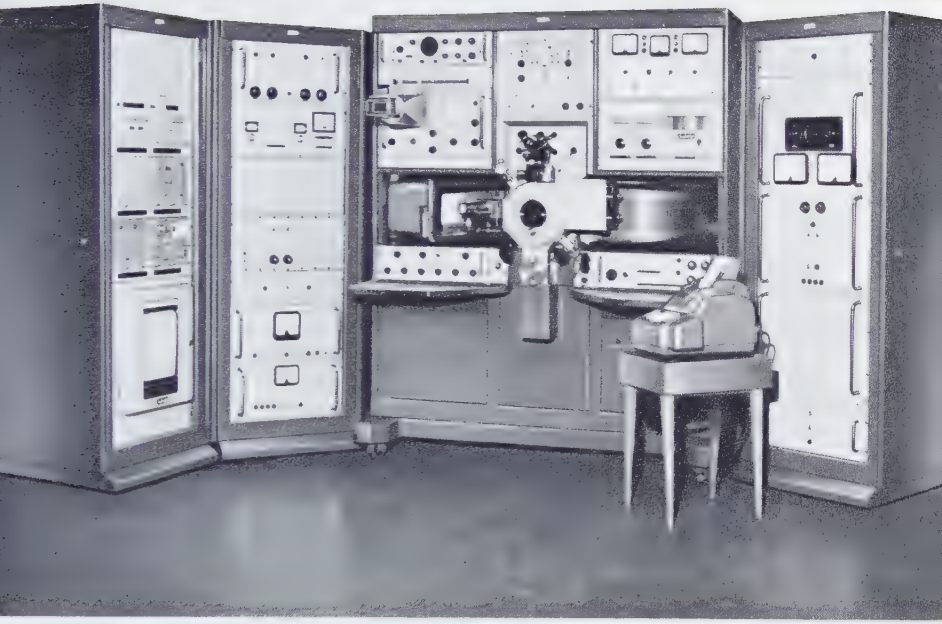
A



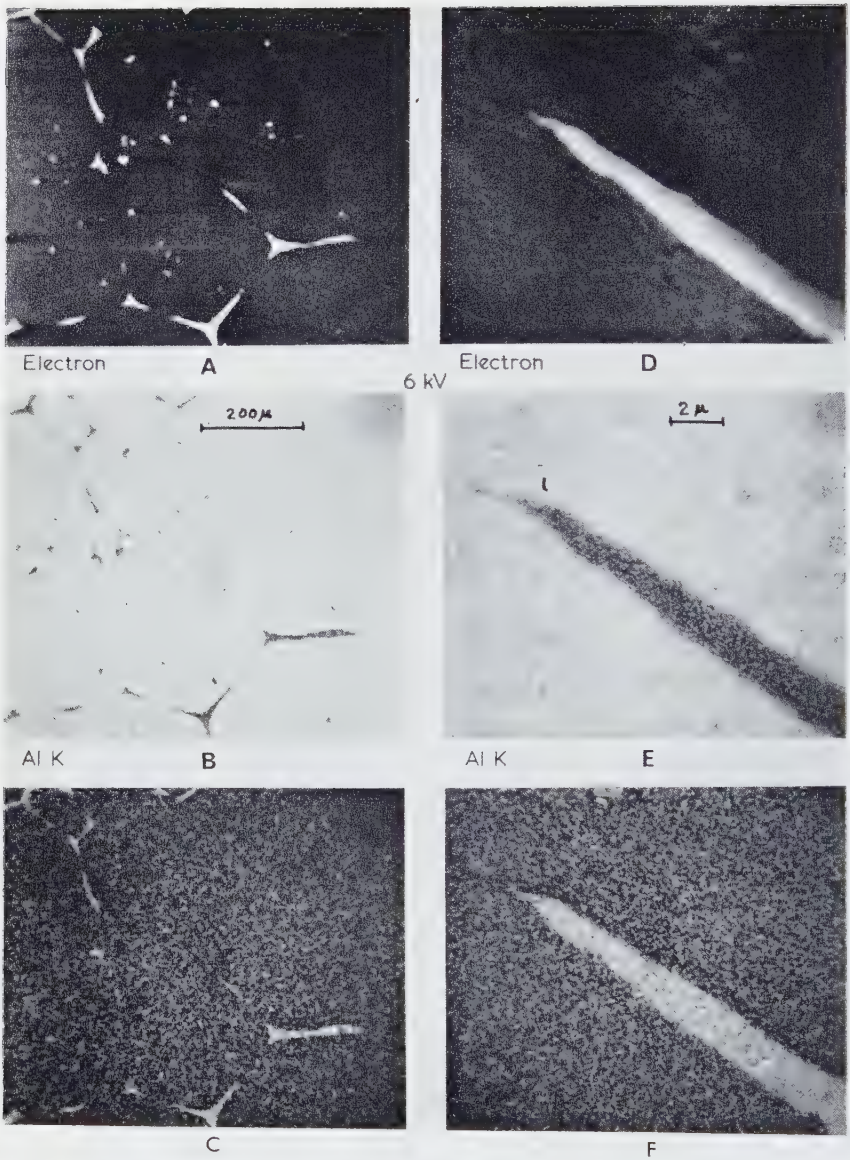
B



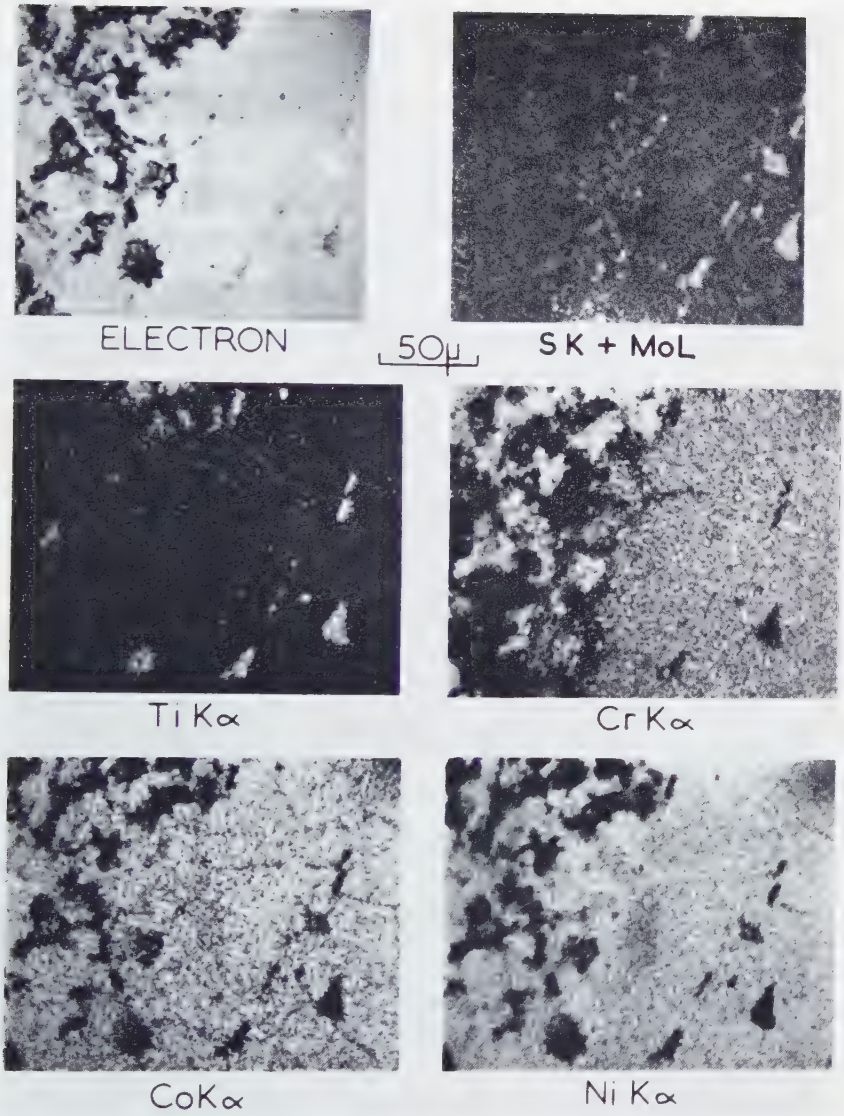
24A and B. Metal oxide silicon transistors, imaged in the scanning electron microscope, showing changes in the relative contrast of the source (S), 'gate' (G) and 'drain' (D) electrodes when the voltage applied to D is 0 volts and that to G is in (A) 0 volts and in (B) −6 volts (Plate A is ×64 and plate B is ×410). (*Dr. P. R. Thornton, University College, Bangor, and Dr. A. D. G. Stewart, Cambridge Instrument Company*)



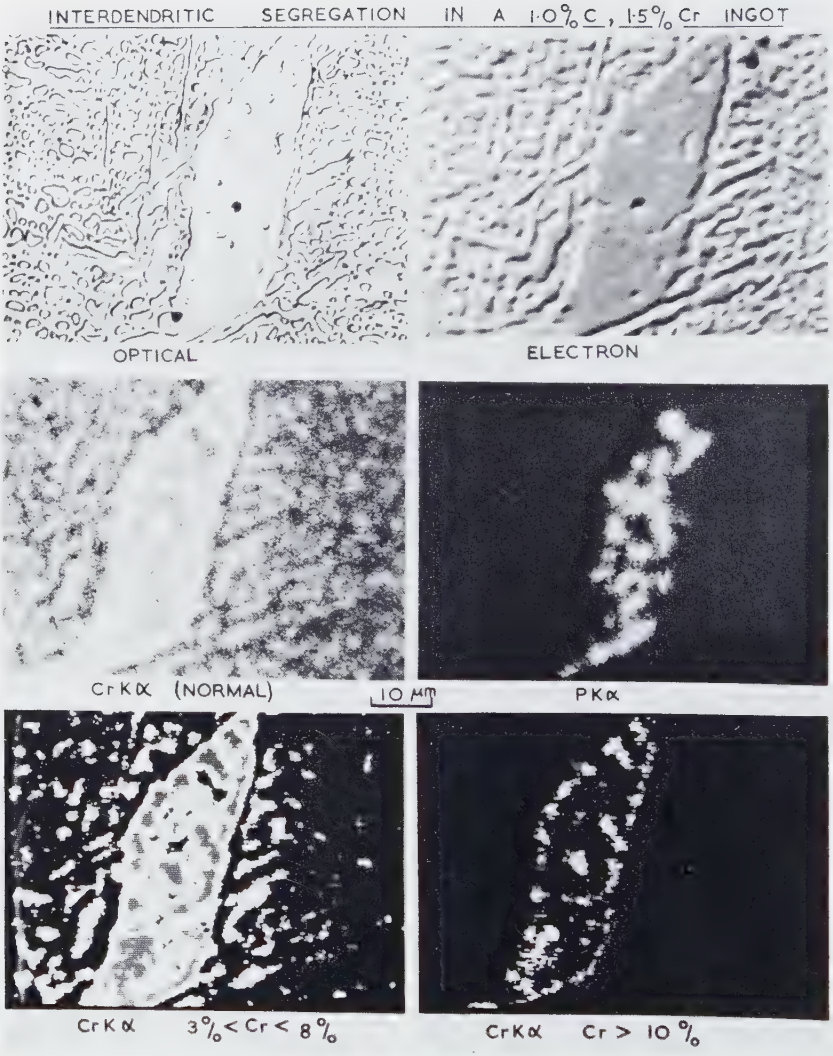
25. Scanning electron probe microanalyser, 'Geoscan' model. The axis of the electron beam is horizontal and directed at the centre of the large black disc in a white surround in the middle of the picture, on either side of which are the X-ray spectrometers (in the curved tanks); on the stool, an automatic typewriter for printing out data from the counter system. (*Cambridge Instrument Company*)



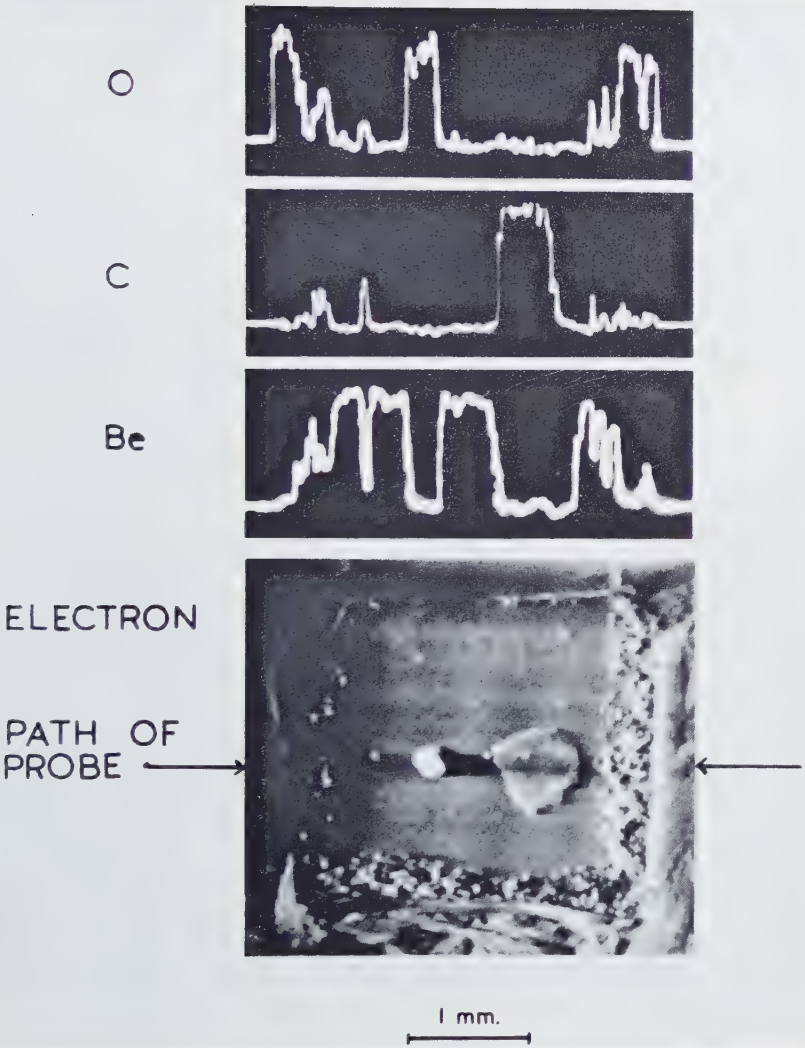
26. Grain boundaries (rich in tin) in an aluminium-6 per cent tin alloy, imaged in the scanning microanalyser.
A and D: Electron images.
B and E: X-ray images formed with aluminium K-radiation.
C and F: X-ray images formed with tin L-radiation.
(A, B, C × 70; D, E, F × 3,300). (*Dr. P. Duncumb, Cavendish Laboratory, Cambridge University*)



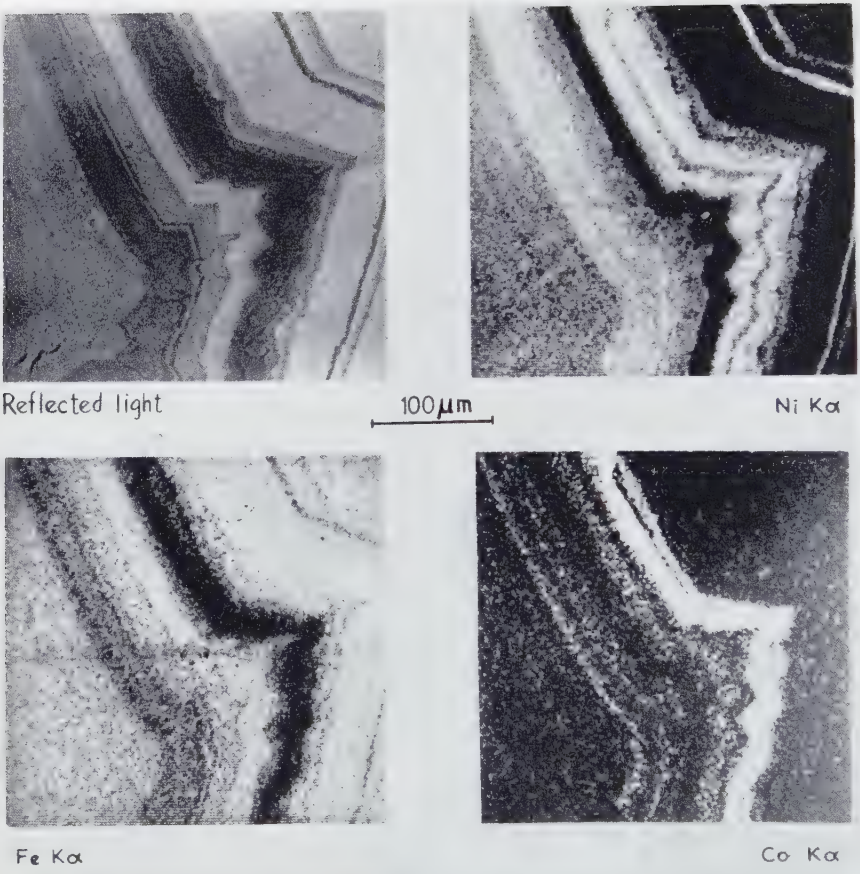
27. Comparison micrographs of a high-temperature alloy taken with the scanning microanalyser, and formed with electrons or the characteristic X-rays of different elements as indicated ($\times 240$). (*Thornton Research Centre of the Shell Company*)



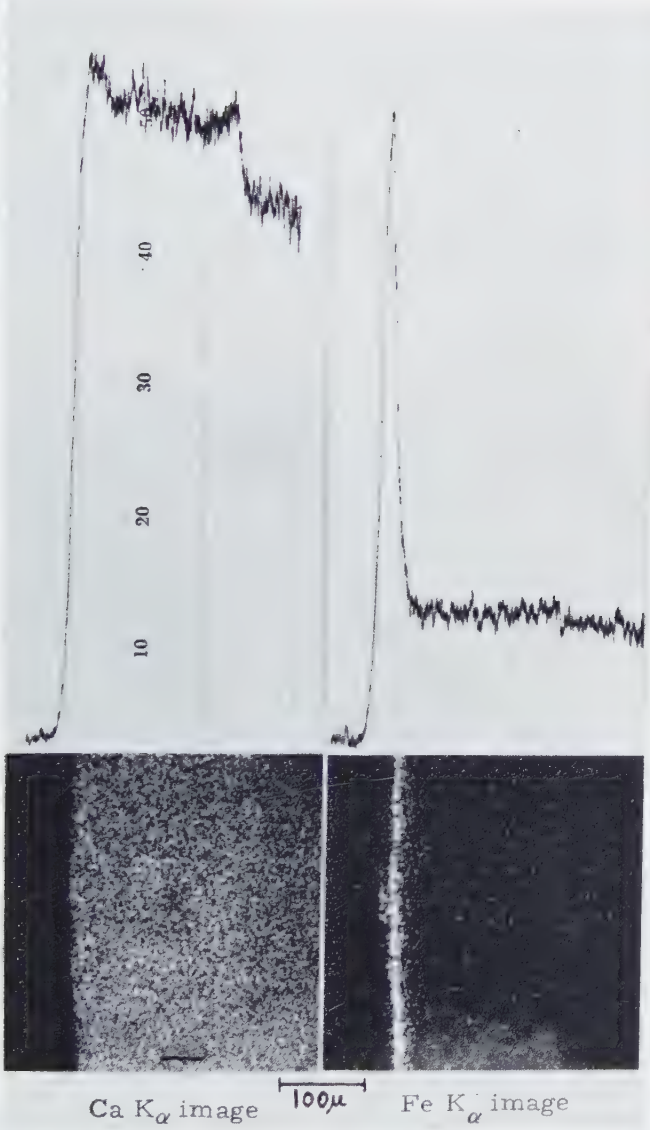
28. Concentration mapping with the scanning microanalyser; chromium distribution imaged at three different levels of concentration by electronic gating. Phosphorus X-ray image to show its association with chromium. Optical and electron images for comparison (×750). (*Dr. D. A. Melford, Tube Investments Research Laboratories, Hinxton, Nr. Cambridge*)



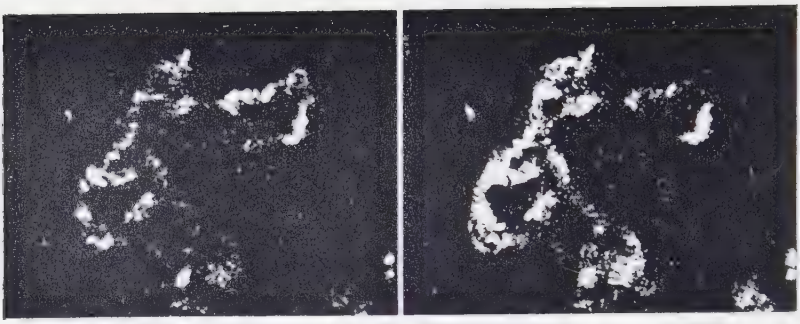
29. Pen traces of X-ray intensity showing relative concentrations of oxygen, carbon and beryllium along a line across an oxidised sample of beryllium, shown in the electron image below, on which was placed a spot of graphite as carbon standard and one of lithium hydroxide (white spot) as oxygen standard ($\times 15$). (*Dr. R. M. Dolby, Cavendish Laboratory, Cambridge University*)



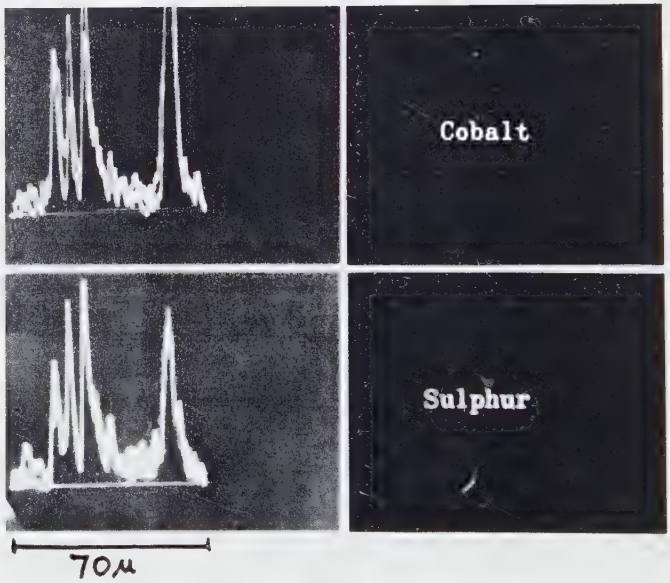
30. X-ray images from the scanning microanalyser showing distributions of nickel, iron and cobalt in a mineral, bravoite; compare with text-figure 50 ($\times 150$). (*Dr. G. Springer and Dr. J. V. P. Long, Department of Mineralogy and Petrology, Cambridge University*)



31. Scanning microanalyser images and pen traces of X-ray intensity showing distribution of calcium and iron across a section of rat tooth; the iron is concentrated in the surface enamel (×110). (*Dr. A. Boyde, Dr. V. R. Switsur and Dr. R. W. Fearnhead, London Hospital Medical School and Cavendish Laboratory, Cambridge University*)



A B



C

D

32. Distribution of an enzyme (alkaline phosphatase) in a section of rat kidney, as revealed by a staining method which deposits cobalt sulphide.
A and B: Images formed with X-rays from cobalt and sulphur ×200. C and D: Corresponding traces of X-ray intensity across the section (×360). *Dr. A. J. Hale, Department of Pathology, St. Thomas's Hospital, London, and Dr. V. R. Switsur, Cavendish Laboratory, Cambridge University*)

The author is Reader in Electron Physics in the University of Cambridge and Vice-President (and past President) of the Royal Microscopical Society. Since 1946 he started and has gradually built up the Electron Microscopy Section of the Cavendish Laboratory, the only unit of its kind in a university in this country. Interest in the biological uses (and limitations) of the electron microscope led him to explore the possibilities of X-rays for microscopy. Together with W. C. Nixon, the projection X-ray microscope was invented and developed at the Cavendish, and together with P. Duncomb, the scanning electron probe microanalyser similarly. Both these instruments are now in commercial production—the microanalyser in particular finding wide-spread uses in metallurgy, mineralogy and (to some extent) in biology.

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